ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTES OF HEALTH

1958

NATIONAL HEART INSTITUTE

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PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

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ANNUAL REPORT OF
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NATIONAL HEART INSTITUTE
1958

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REPORT OF 1958 NHI PROGRAM ACTIVITIES
REFERENCE SHEET

Laboratory	Proj. #'s	Sum. Statem. Page #'s
General Medicine and experimental Therapeutics Branch	21 - 58	54 - 62
Section on Clinical Endocrinology	44 - 58	54 - 56
Section on Experimental Therapeutics	21 - 27	56 - 58
Section on Cardiodynamics	28 - 43	59 - 62
Gerontology Branch	59 - 99	66 - 70
Heart Disease Control Program	6 - 20	-
Laboratory of Cardiovascular Physiology	100 - 110	21 - 26
Laboratory of Cellular Physiology and Metabolism	111 - 157	1 - 16
Section on Cellular Physiology	111 - 125	1 - 6
Section on Enzymes	126 - 136	6 - 11
Section on Metabolism	137 - 157	11 - 16
Laboratory of Chemistry of Natural Products	158 - 173	17 - 20
Laboratory of Chemical Pharmacology	174 - 225	39 - 51
Laboratory of Clinical Biochemistry	226 - 233	52 - 70
Laboratory of Kidney and Electrolyte Metabolism	234 - 248	27 - 33
Laboratory of Technical Development	273 - 286	34 - 38
Office of the Director	1	-
Surgery Branch	249 - 272	62 - 65
Technical Services	2 - 5	-
Biometrics Research Section	2	-
Heart Information Center	3	-
Epidemiology Section (Framingham)	4	-
Grants and Training Branch	5	-

National Heart Institute

Summary of Intramural Research Activities

1958

Laboratory of Cellular Physiology and Metabolism -- Section on Cellular Physiology

The following major research projects have been under investigation in the Section on Cellular Physiology during the past year:

1) The correlation of structure with enzymatic function in the ribonuclease molecule.
2) The mechanism by which genetic information is utilized in the biosynthesis of protein molecules, with particular emphasis on the biosynthesis of the proteins of bacteriophage.
3) Studies on the structure of fibrous proteins, including myosin, fibrinogen and collagen.
4) The biosynthesis of proteins in the hen's oviduct.
5) Oxidative phosphorylation and electron transport.
6) Structure and metabolic role of lipoproteins.

1) The covalent structure of ribonuclease is now essentially completely known and it is possible to examine, in a rational way, the relationships between specific parts of the molecular structure and the enzymatic activity of this protein. Three major approaches to this problem have been employed. In the first, a systematic study of the reduction of disulfide bridges in ribonuclease has revealed that at least one, and possibly as many as three, of the four disulfide bridges in the molecule may be cleaved by reductive cleavage to the SH- form without loss of all the enzymatic activity. One of these bridges, namely, that connecting half-cystine #1 with half-cystine #6 (counting from the N-terminal end of the molecule) may be opened, and the SH groups so formed stabilized by conversion to their carboxymethyl derivatives, without loss of activity. It is certain that at least one more bridge may be opened with impunity and its identity is now under investigation by the "fingerprinting" technique to be described below.

The finding of major significance is that ribonuclease, in which three of the four disulfide bridges have been reduced (this derivative having about 10-15% the activity of the native enzyme), may be reoxidized with molecular oxygen with the complete disappearance of SH groups, and

with the regeneration of 100% of the original activity. Physical studies show that this reformed, native-like molecule contains even more intramolecular coiling than the original substance, in spite of its normal activity. The results suggest that the presence of the single remaining disulfide bridge is sufficient to direct the reformation of a second critical bridge, but that the third and fourth disulfide bond may reform in an abnormal way but without a deleterious effect on function. The arrangement of the disulfide bridges in the reoxidized molecule are now under investigation.

Of major importance in these studies and in a number of the other activities of the laboratory has been the development of a simple and reproducible technique for the rapid, qualitative study of the general features of structure in an unknown protein. Samples of protein are first digested with proteolytic enzymes. The resulting peptide mixture is then separated into its components by successive paper chromatography and electrophoresis to yield a characteristic pattern of peptide spots. These may be located by staining with ninhydrin and the peptides so located may be isolated and analyzed for their amino acid composition.

This technique has been of considerable use in connection with studies on ribonuclease discussed above. Thus, for example, fingerprints have been prepared of digests of bovine, ovine and porcine ribonucleases of pancreas tissue and the resulting peptides have been separated, analyzed and compared with one another. Certain regions of the molecule have been found to show differences from species to species and, in one instance, sheep ribonuclease, a lysine residue characteristic of the beef enzyme, has been replaced by glutamic acid. This major species difference suggests, at once, that the particular area of the molecule in which this variation occurs cannot be critically involved in the binding or hydrolysis of ribonucleic acid. Similar species studies are now in progress on a ribonuclease from spinach leaves and it is hoped to extend the work to ribonucleases from Aspergillus, E. coli, and Streptococcus.

The location of the active center in ribonuclease is also being investigated by the application of controlled proteolytic digestion. Some enzymes remove small portions of the peptide chain with inactivation whereas others attack the chain without inactivation. Both types of events yield valuable information as to the location of the functional constellation of amino acids.

The approach outlined above will, it is hoped, eventually give sufficiently specific information to enable us to begin a synthetic approach to the active center of this enzyme.

2) Recent advances in genetic techniques, particularly with microorganisms and viruses, have made it clear that genes consist of a large number of loci, probably of an order of size corresponding to only very few nucleotides in the DNA molecule. Geneticists have been able to construct maps of the genetic material of a number of organisms in which a large number of such loci have been pinpointed within a single functional gene, presumably controlling the biosynthesis of a single protein.

One such study has involved the T-even bacteriophages and a genetic map for the host range specificity function has been constructed by Streisinger and his colleagues. The protein in the coat of the virus particle which is responsible for host range specificity has been purified in this laboratory to a very high degree and samples of this protein isolated from various mutants which have undergone mutations in different positions along the corresponding region of the genetic material are now being subjected to "fingerprinting." This study is aimed at determining whether or not there exists a direct correspondence between changes in the chemical structure of the DNA "chromosome" of the phage and the amino acid sequence of the protein whose synthesis is under the control of this region. Another protein in the coat of the virus particle has to do with the penetration of the viral DNA into the host bacterial cell. This protein has been identified as a lysozyme and has been shown to be similar in many of its properties to the corresponding enzyme in egg white and in various other tissues. Lysozyme has been isolated in reasonable quantities from bacteriophage T2 and from lysates of E. coli produced by phage infection. A search is now under way for a technique by which the genetic loci in bacteriophage T2 may be mapped so that the study of correspondence between genetic information and lysozyme structure can be undertaken. In parallel with these studies, a systematic investigation of the structure of egg white lysozyme is being pursued in order to obtain a baseline for comparison of the enzyme from the bacteriophage. These studies also involve the fingerprinting technique described above.

(3) As a consequence of studies on the three-dimensional structure of polyproline and of proline-glycine copolymers, information was gained which has contributed considerably to the understanding of the

behavior of collagen, which is a protein rich in proline. The studies on the synthetic substances indicated that they underwent cis-trans isomerization at a critical temperature which caused a large change in the length and folding of the molecule. Similar physical behavior is exhibited by a variety of collagens. Of particular interest is the finding that the process of conversion of collagen to gelatin also seems to involve cis-trans isomerization at the proline-proline bonds and that the process of conversion of collagen to gelatin, and its reversal, may be dependent upon such isomerization as well as on that contributed by the rupture and reformation of hydrogen bonds.

The studies on collagen have led Dr. Harrington and his colleagues into the more general problem of the structure of fibrous proteins. These studies involve the use of specific degradative enzymes as probes of secondary structure. In the case of collagen for example, it has been found that collagenase is highly specific for the sequence X-gly-pro. With the use of this specific enzymatic reagent a limited number of cleavages may be induced and, through the analysis of the kinetics of the cleavage process, much information may be gained relating to the distribution of "amorphous" and "crystalline" regions. Amorphous regions not stabilized by systematic hydrogen-bonded structures are more susceptible to digestion than crystalline portions into which the attacking enzyme cannot, as easily, penetrate. The concept of fibrous protein structure as a composite of amorphous and crystalline portions has also been examined with myosin. It has been found that trypsin digestion of myosin proceeds in a discontinuous fashion, some 60 peptide bonds in the molecule being rapidly digested, whereas several times as many bonds are slowly digested. Physical studies support the idea that the amorphous and crystalline regions alternate along the rod-like myosin molecule. The studies, incidentally, have made completely untenable the earlier conclusions of Szent-Gyorgyi on the production of so-called light and heavy meromyosin and indicate that these "subunits" are probably experimental accidents. Ultracentrifugal studies on myosin in 5 M guanidine solution indicate that myosin may consist of three identical polypeptide strands coiled around one another, much like the three strands in collagen. The three strands are thought to be identical since application of the fingerprinting technique to trypsin digests yields far too few peptide fragments to allow the presence of three chemically different component polypeptide strands. Experiments are now in progress to determine some of the covalent features of myosin, particularly the nature of its end groups and the mode of bonding which stabilizes the three-stranded structure.

4) Studies on the mechanism of protein biosynthesis in the hen's oviduct are being continued. Dr. Hendler has made considerable progress in the isolation and characterization of the interesting lipid-soluble substances which appear to be good candidates as intermediates in protein biosynthesis in this tissue. Five distinct fractions can be separated by the use of aluminum silicate columns and countercurrent distribution and the fractions contain very large amounts of radioactivity when isolated from tissue which has been incubated with radioactive amino acids. These studies are of great interest in view of the lipid nature of the endoplasmic reticulum which is so likely to be involved in the assembly of protein molecules. In the course of these studies Dr. Hendler has also reinvestigated the technical problems involved in the counting of C^{14} and has developed interesting modifications of the theories originally proposed by W. Libby and others for the self-absorption phenomenon.

5) Drs. Kielley and Bronk have continued their studies on the nature of oxidative phosphorylation along the lines of last year's work. Thyroxin and thyroxin analogues having hormone activity have been shown to concentrate oxidation without altering phosphorylating efficiency. At higher concentrations and following preincubation, uncoupling was observed. The observations have suggested that the uncoupling effect is a secondary one but it is still uncertain whether the acceleration of oxidation can be regarded as physiologically significant. A number of Vitamin K and Vitamin E analogues have also been examined in the submitochondrial system. Some of these caused marked acceleration of phosphorylation and are being further investigated.

6) Dr. Rodbell has examined the alpha and beta lipoprotein molecules in parallel with the proteins which are present in chylomicrons. One of the major components in chylomicrons has been shown, by the fingerprinting method, to be identical with the high-density lipoprotein of plasma. Studies with C^{14}-labelled amino acids have indicated that the alpha lipoprotein of plasma is not in equilibrium with the corresponding chylomicron protein and that the chylomicron synthesis must involve a separate pool of this particular protein substance.

The high-density lipoproteins may be made soluble in aqueous systems by removal of the lipid components. Studies are under way to investigate the "re-synthesis" of alpha lipoproteins from its component parts. Dr. Rodbell, together with Dr. Fredrickson of the Section on

Metabolism, is also actively investigating the metabolic origin of the chylomicron proteins and high-density lipoproteins using radioactive techniques.

--Section on Enzymes

During the past year the Section on Enzymes has been concerned with studies on the enzymatic mechanisms of the following biochemical processes: 1) the metabolism of heterocyclic compounds; 2) the metabolism of three carbon compounds; 3) anaerobic oxidative phosphorylation and electron transport; 4) cellular differentiation and protein synthesis; 5) the metabolism of isoprenoid derivatives; 6) homoserine metabolism.

1) The metabolism of heterocyclic compounds. Riboflavin degradation (E. R. Stadtman, P. Smyrniotis, T. Miles). Several intermediates in the oxidation of riboflavin to carbon dioxide and ammonia have been isolated from the culture medium of an aerobic bacterium grown on riboflavin as its major source of carbon, nitrogen and energy. Detailed chemical and enzymatic analysis of these compounds reveals the existence of at least two major pathways of riboflavin dissimilation. One mechanism involves an initial oxidative cleavage of the pyrimidine ring (ring C) with the formation of stoichiometric amounts of 1-ribityl-2, 3-diketo-1, 2, 3, 4 tetrahydro-6, 7-dimethylquinoxaline (compound I), urea, and a one carbon derivative (probably CO_2). This is a curious transformation since all of the most reasonable postulated intermediates have been synthesized, tested and found not to be metabolized. Compound I is subsequently converted to a second closely related compound (compound II) by a synthetic reaction which involves removal of two oxygen atoms from the ribityl side chain and the addition of two carbon atoms and one nitrogen atom to the side chain. The exact structure of compound II is yet undetermined. Compound II is subsequently converted by a series of unknown reactions to 3, 4 dimethyl-α-pyrone-5-carboxylic acid which, in turn, is oxidized to CO_2. A second pathway of riboflavin breakdown is indicated by the isolation of other metabolites which are oxidized to CO_2 and NH_3 without the intermediary formation of any of the above mentioned substances. Preliminary evidence indicates that this pathway involves an initial attack on ring A of the riboflavin molecule with the formation of a pteridine derivative. One such metabolite has been found to replace riboflavin for growth of a riboflavin requiring strain of lactobacillus, suggesting that it may be an intermediate in riboflavin biosynthesis.

2) The metabolism of three carbon compounds.

(a) Propionic acid oxidation (Dr. Vagelos). A new pathway for the biological oxidation of propionic has been elucidated in studies with cell-free enzyme preparations of the anaerobic bacterium C. kluyveri. This mechanism involves the intermediary formation of propionyl CoA, acrylyl CoA, β-hydroxy-propionyl CoA, malonyl semialdehyde CoA, and malonyl CoA. The fate of malonyl CoA is still uncertain but some results indicate that it may be decarboxylated to acetyl CoA and CO_2. These results supplement those previous findings (reported last year) of lactoyl CoA and β-alanyl CoA as activated intermediaries in the metabolism of propionic acid. Proof for the biological formation of these activated three carbon compounds directs attention to their possible role in the energy metabolism of diverse biosynthetic processes.

(b) The metabolism of pyruvate and alanine (Drs. H. Goldfine and E. R. Stadtman). Enzyme preparations of C. propionicum catalyze the conversion of alanine to a mixture of acetic acid, CO_2, NH_3 and propionic acid. Detailed studies of this fermentation have revealed the surprising fact that pyruvate is not an intermediary in the oxidation of alanine. During the course of these studies it was discovered that with cell-free extracts pyruvate is utilized for the synthesis of a major metabolite which has been isolated and tentatively characterized as a hydroxy-dicarboxyamino acid (probably γ-amino-γ-methyl-glutamic acid).

Studies are in progress on the mechanism of this reaction and the role of the new amino acid metabolite in the metabolism of this organism.

3) Anaerobic oxidation phosphorylation and electron transport.

a) The oxidation of diphosphopyridine nucleotide by crotonyl Coenzyme A (Drs. E. Brown and E. R. Stadtman). From purely thermodynamic considerations, it has been postulated that the energy for growth of the anaerobic bacterium C. kluyveri is derived from the reduction of crotonyl-CoA to butyryl CoA by reduced diphosphopyridine nucleotide. This reaction is associated with a standard free energy change of about -14,000 calories. A recent report has claimed that in soluble cell free preparations of C. kluyveri, phosphorylation is coupled with the above oxido-reduction process. Extending our own studies in this biochemical reaction, the occurrence of phosphorylation in the anaerobic metabolism of crotonyl CoA and related thiolester derivatives has been confirmed;

however, the results suggest that the phosphorylation is not associated with the oxidation of diphosphopyridine nucleotide but most probably is the result of a dissimulation of crotonyl CoA to butyryl CoA and acetyl CoA followed by the formation of ATP from the latter compound via acetyl phosphate. An alternative experimental approach which avoids the possibility of phosphorylation via acetyl phosphate is under investigation in a continuing effort to study phosphorylation coupled to anaerobic electron transport.

(b) Reductive deamination of amino acids (Dr. T. C. Stadtman). Studies on the mechanism of ATP formation associated with the enzymatic reduction of glycine to acetate and NH_3 have been continued. As judged by a number of criteria such as marked sensitivity to antimycin A, inactivation by menadione and related simple naphthoquinones, inactivation by irradiation or solvent extraction procedures, and activation of aged or solvent-treated extracts by vitamin E, it is believed that a quinone (possibly a vitamin E derivative) is involved in the overall process. The incidental discovery that these enzyme preparations contain a menadione-dependent phosphatase suggests the possibility that inhibition of the phosphorylation process by menadione is the result of an uncoupling action of this compound when substituted for the natural quinone intermediate.

The bioenergetics of reductive deamination is also being investigated (Dr. T. C. Stadtman and Mr. Hardman) in organisms that catalyze specific fermentations of γ-amino butyric and Δ-amino valeric acids to mixtures of ammonia and lower fatty acids. Studies with cell-free extracts of the γ-amino-butyric acid fermenting organism have shown that reduction to butyrate proceeds via succinicsemialdehyde and γ-hydroxybutyric acids as intermediates and the enzymes involved in the metabolism of these compounds have been partially purified and their Co-factor requirements established. In future studies attention will be directed to elucidation of the energy-yielding reactions which also appear to be present in soluble extracts.

(c) The metabolism of N-onium compounds (Drs. H. Hayward and T. C. Stadtman). The conversion of N-onium compounds to primary or secondary amines is associated with a relatively large change in standard free energy. In order to determine if the potential energy of such reactions can be used for biological synthesis, a study was undertaken to investigate the fermentation of choline by an obligate anaerobic

clostridium capable of utilizing this substance as its sole energy and carbon source. Cell-free extracts of this organism have been shown to catalyze the conversion of choline to one mole of trimethylamine and one-half mole each of acetate and ethanol. Extracts of this organism were found to contain a cytochrome pigment which is spectrally similar to animal cytochrome C. This discovery is the first exception to the previous generalization that clostridia do not contain cytochrome pigments. Although the exact role of this pigment in the metabolism of choline is not yet known, it is of special interest that in order to serve as an electron carrier under the anaerobic conditions of metabolism, it must function at a redox potential well below that of the animal cytochrome system. The biochemistry of this pigment and the possibility that anaerobic dissimulation of choline is coupled with phosphorylation are immediate topics of further study.

4) Homoserine metabolism (Dr. Flavin). O-phospho-homoserine has been previously demonstrated to be an obligatory intermediary in the isomerization of homoserine to threonine in certain microorganisms. From the mechanistic point of view the role of a phosphate ester in such an isomerization is of special interest since there is no established precedent for such a reaction in metabolism. It is of further interest because it may be analogous to, and serve as a useful model for, an early step in the biosynthesis of steroids; (viz. in the conversion of mevalonic acid pyrophosphate to Δ^3-isopentenol pyrophosphate).

The enzyme catalyzing the conversion of phosphohomoserine to threonine and orthophosphate has been extensively purified from extracts of Neurospora and was shown to require pyridoxal phosphate as a coenzyme. Studies of this reaction in tritium-labeled water reveal that no significant incorporation of tritium into threonine occurs. This eliminates from further consideration one postulated mechanism involving the intermediary formation of an olefinic derivative such as vinylglycine.

(d) The reduction of diphosphopyridine nucleotide (DPN) by molecular hydrogen (Drs. S. Kinsky and E. R. Stadtman). Two heat-stable coenzymes are involved in the reduction of DPN by molecular hydrogen as catalyzed by soluble extracts of C. kluyveri. One of these coenzymes was identified as flavine adenine dinucleotide (FAD). Purification of the second coenzyme is still in progress. As judged by behavior on ion exchange resins, electrophoretic mobility and adsorbability on charcoal, etc., it appears to be a strongly anionic substance probably of nucleotide nature. The discovery that FAD is an obligatory coenzyme

in the reduction of DPN by hydrogen is of special interest since the standard redox-potential of free FAD is well above that of either hydrogen or DPN. Therefore, from thermodynamic considerations it seems probable that in the hydrogenase system the potential of the FAD is markedly reduced perhaps by combination with the apo-enzyme. The probable existence of such low-potential flavin systems requires a re-evaluation of the concept that flavin enzymes, because of their high potential, are involved only as electron carriers in terminal respiration.

5) The biochemistry of cellular differentiation and protein synthesis (Dr. B. Wright). The dramatic changes in the morphology and metabolism of the slime mold during the process of cellular differentiation are associated with marked changes in enzyme composition and in concentration of the free amino acid pool. A kinetic analysis shows that as differentiation proceeds, first, the size of the free amino acid pool decreases to one-third its initial value, then the alcohol-soluble proteins decrease and finally between the slug and fruit stages, the remaining protein fraction decreases. The greatest proteolytic activity occurs after the free amino acid pool reaches a low level, at which time many enzyme activities are at their highest. The results are consistent with the working hypothesis that a decrease in amino acid pool, brought about by starvation, may initiate endogenous protein breakdown.

Evidence has been obtained showing that acrasin, the chemotactic steroid-like hormone which is needed to initiate aggregation of amoebae during differentiation is not involved as a coenzyme in the DPNH-TPNH transhydrogenase system nor does it have any direct effect on terminal respiration.

6) The metabolism of isoprene derivatives. Citronellol is a low molecular weight compound (2 isoprene units) and it serves as an ideal model compound to study isoprenoid degradation. Therefore in order to facilitate studies on the biochemistry of polyisoprene metabolism, an aerobic bacterium was isolated from soil enrichment that can utilize citronellol as its sole carbon and energy source. Studies with cell-free extracts of this bacterium were found to contain all of the enzymes previously established in the biosynthesis of acetoacetate, β-hydroxybutyrate, and β-hydroxy-β-methylglutaryl CoA, and in terminal respiration by the TCA cycle. However, during the oxidation of acetate by cell suspensions up to 25% of the acetate carbon is utilized for the biosynthesis of a yellow substance which has been isolated and tentatively

identified as a terpene. It is further demonstrated that during the oxidation of citronellol or isovaleric acid, extensive incorporation of $C^{14}O_2$ into acetate and β -hydroxymethylglutaric acid occurs. The detailed mechanisms of citronellol catabolism and the biosynthesis of the terpene-like compound formed from acetate is being pursued further.

--Section on Metabolism

The research activities of the Section on Metabolism for the year 1958 are best described under several categories:

1) studies on the basic physiology of fat absorption and transport;
2) studies of the factors controlling lipoprotein levels, including in vitro studies of lipoprotein biosynthesis and the effects of various agents such as dietary fats;
3) studies on the mechanisms of protein biosynthesis;
4) studies of clinical and experimental nephrosis, including immunochemical studies;
5) basic studies on protein structure;
6) studies on plasma protein degradation and excretion.

1) Studies on the basic physiology of fat absorption and transport. Previous studies in this laboratory have established the importance of unesterified fatty acids (UFA) in the transport of fat from tissue depots to sites of oxidation. It has now been shown in man that there is very little difference in the rates at which different fatty acids (palmitic, oleic and linoleic acids) are converted to carbon dioxide. Exercise markedly increases the rate of conversion of circulating UFA to CO_2 (6-10 fold).

The previously reported action of epinephrine in elevating serum UFA concentrations has been further studied. The elevation of serum UFA following epinephrine is not maintained even when large amounts of epinephrine are administered intravenously. The return of UFA levels toward normal occurs at about the same time the blood glucose levels rise. In separate studies it was shown that administration of large amounts of glucose could prevent the epinephrine-induced UFA response. Studies on adipose tissue in vitro show that this is a direct

effect, that is, glucose in high concentrations prevents the stimulation of UFA release from adipose tissue. In view of these findings and the data in the literature implicating glycogen of adipose tissue in fat mobilization, studies have been initiated in an attempt to demonstrate that the action of epinephrine on adipose tissue is mediated through an effect on glycolysis. Preliminary findings tend to support this hypothesis.

The question of the relative importance of serum lipoproteins and serum unesterified fatty acids in the transport of fat under various conditions remains to be evaluated. An attempt is being made to block UFA transport by introducing a fatty acid analogue which cannot be metabolized but which is transported as an albumin complex. 3, 3-dimethyl-13-phenylmyristic acid was synthesized and prepared in radioactive form by tritiation. It has been shown that this molecule is not metabolized at any significant rate and that it competes for binding sites on albumin quite effectively (binding constant similar to that of palmitic acid). This fatty acid persists in the circulating blood for much longer periods than does palmitic acid and a very high fraction persists as UFA within the tissues. Larger amounts of this analogue are being prepared to permit loading of an animal with enough analogue to block UFA transport.

The method of vapor phase chromatography for analysis of fatty acid mixtures has been extensively studied in collaboration with the Laboratory of Technical Development. A detection device based upon a new principle has been perfected and is in operation and a simple method for introducing small samples has been devised.

2) Studies of the factors controlling lipoprotein levels, including in vitro studies of lipoprotein biosynthesis and the effects of various agents such as dietary fats. Very little is known about the homeostatic mechanisms regulating serum lipoprotein and cholesterol levels. Because of the many variables involved in whole animal studies it was considered desirable to devise in vitro systems for the study of lipoprotein synthesis. For the first time the synthesis of lipoproteins by liver slices has now been demonstrated. Incorporation of radioactive amino acids into the protein moiety of lipoproteins has been accomplished. The identity of these lipoproteins with serum lipoproteins has been conclusively shown in the case of the α_1-lipoproteins and presumptive evidence obtained in the case of β_1-lipoproteins. Rat liver slices both synthesize and secrete lipoproteins in a simple inorganic medium. Biosynthesis of lipoproteins has also been demonstrated in isolated perfused

rat liver. These techniques should be valuable in assessing the effects of various agents on rates of lipoprotein synthesis.

Studies of the end groups in the protein moiety of chylomicrons suggest the presence of at least three different proteins. One of these appears to be identical with the major protein component of alpha-lipoproteins. The possible implications of this with regard to fat transport during digestion are being explored.

The effects of dietary fats on lipoprotein levels and on cholesterol degradation and excretion have been studied. The striking effects of unsaturated fats in lowering serum cholesterol levels have been confirmed but it has been noted that almost as great an effect can be obtained by withdrawing fat from the diet. That the effect of unsaturated fats is not dependent upon their content of vegetable sterols has been shown in studies utilizing an especially prepared safflower oil containing only a fraction of a per cent of these vegetable sterols.

During studies of cholesterol degradation and excretion in patients receiving C^{14}-cholesterol intravenously it was noted that a very high fraction of the excreted radioactivity appeared in the feces in the form of neutral sterol. This finding is in apparent conflict with the previously accepted concept that bile acids represent the main excretory form of cholesterol. In six patients the percentage radioactivity excreted as neutral sterol ranged from 35 to 80 per cent with the remainder appearing in the form of bile acids. This discrepancy with earlier results must be due either to the considerable reabsorption of bile acids which occurs or to excretion of cholesterol by the intestinal tract. No consistent change in total cholesterol end product excretion was observed when the nature of the dietary fat was changed from saturated to unsaturated.

Studies in rabbits have shown that the rate of regression of cholesterol-induced atheromata is accelerated when the diet includes corn oil compared to that rate observed when the diet includes coconut oil.

It has been shown that epinephrine, in addition to raising serum UFA levels, causes a rise in serum lipoprotein levels in dogs, maximal at 24 hours. Daily injections of long lasting epinephrine maintains this elevation of serum lipoproteins. The elevation occurs primarily in the β 1-lipoprotein fraction and thus the elevation is primarily in cholesterol

and phospholipids (about 50 per cent above control values). The possible importance of this observation in relation to stress-induced hypercholesterolemia is being given serious consideration.

The use of inhibitors of cholesterol synthesis to effect a lowering of serum cholesterol levels has been further explored. Several compounds structurally related to Δ^4-cholestenone have proved ineffective as inhibitors (2-alpha-methyl-cholestenone, 6-alpha-methyl-cholestenone, sitostenone and 3-methoxy-cholesterol). It has been previously postulated that the effectiveness of Δ^4-cholestenone may depend upon its conversion to dihydrocholesterol. The ineffectiveness of the substituted cholestenone derivatives may possibly be attributable to the inability of the organism to reduce them. Benzmalacene (N-Cl-methyl-2, 3-di-p-chlorophenylpropylmaleamic acid) has been shown to be effective in lowering cholesterol levels in dogs up to 50 per cent, confirming studies by Merck, Sharp and Dohme. Clinical studies on a small number of patients indicate the drug to be effective but gastric irritation and elevation of BSP retention appear to be problems. MER-29 (1-(p-(β-diethylaminoethoxy)-phenyl)-1-(p-tolyl)-2-(p-chlorophenyl) ethanol) profoundly influences cholesterol metabolism in the rat, causing the serum cholesterol level to drop as low as 15 mg%, and decreasing the cholesterol content of the liver by 50%. A non-saponifiable lipid distinct from cholesterol accumulates in the liver of treated animals and the nature of this is under study.

A new method for incorporating lipids into lipoproteins in vitro has been developed. This simple procedure permits the incorporation of labeled compounds into lipoproteins at high specific radioactivity and makes it possible to carry out tracer studies with a better approach to the truly physiological state. In addition it promises to be of value in the study of lipoprotein transport of carcinogens and other trace components.

3) Studies on the mechanisms of protein biosynthesis. Studies on the incorporation of amino acid analogues into crystalline proteins have been completed. The conclusive demonstration of such incorporation has important implications with respect to the specificity of protein biosynthesis. Studies of low molecular weight peptide derivatives occurring in rat and lipid liver are continuing. A large family of low molecular weight peptides associated with an acidic component, probably nucleotide in nature, has been demonstrated by means of chromatography

on cellulose columns. Conclusive proof that these are involved in protein biosynthesis has not yet been obtained.

In the course of these studies a method for the radioassay of tritium labeled proteins in the liquid scintillation spectrometer was developed. A more general method for counting aqueous solutions in the liquid scintillation spectrometer has been found and is being further explored to make it routine.

4) Studies of clinical and experimental nephrosis, including immunochemical studies. The lipoprotein patterns in patients with the nephrotic syndrome fall into several distinct categories. Contrary to the general clinical impression, there are a significant number of nephrotic patients in whom the elevation of serum lipids is primarily in the β_1-lipoprotein fraction rather than in the very low density lipoprotein fractions. There appears to be a spectrum of lipoprotein patterns and during therapy with either steroids or albumin the lipoprotein pattern is observed to change from one type to another. These findings cast doubt on the hypothesis that the nephrotic suffers primarily from an inability to convert very low density lipoproteins to higher density lipoproteins. While the effectiveness of intravenous serum albumin in lowering the lipoprotein levels in nephrotic patients has implicated albumin specifically, experimental studies in the rat show that dextran is to some extent able to duplicate these effects of albumin and this raises question as to the specificity of the albumin molecule in this respect. Attempts to demonstrate antibodies to human kidney in the serum of patients with renal disease have been negative. On the other hand, using the same tannic acid hemagglutination test, it has been possible to confirm the presence in the sera of patients with thyroiditis of antibodies against human thyroglobulin. Rabbits immunized with rabbit nucleoprotein extracts develop antibodies both against these homologous nucleoproteins and against human nucleoproteins.

Forty-five patients with the nephrotic syndrome have now been treated with adrenal steroids and partial or complete remissions have been obtained in almost two-thirds of these cases.

5) Basic studies on protein structure. Kinetic studies of the digestion of myosin by proteolytic enzymes reveals two distinct rates. Simultaneous study of the optical properties of the molecule suggest that the portions of the molecule not involved in helix formation are the parts rapidly digested. Preliminary studies suggest a similar phenomenon in the case of collagen.

The gelation of proteins by formaldehyde was studied with the aid of radioactive formaldehyde. It was shown that there was stoichiometry between the number of formaldehyde molecules taken up by the protein and the number of lysine epsilon amino groups blocked during the reaction. These studies point to the formation of methylene bridges between lysine residues as the major mechanism of gel formation.

6) Studies on plasma protein degradation and excretion. The clinical entity of hypercatabolic hypoproteinemia has been recognized in a number of patients who show no apparent basis for their hypoproteinemia. Studies with I^{131} labeled albumin and, more recently, with I^{131} labeled polyvinylpyrrolidone (PVP) have demonstrated that in many of these patients there is a considerable loss of albumin into the intestinal tract. In some cases this has correlated with a clinical lesion such as regional enteritis or ulcerative colitis but in others the leakage does not correlate with manifest intestinal disease. The nature of this syndrome is being further explored.

Laboratory of Chemistry of Natural Products

The following sections summarize work in this laboratory.

A. Isolation Studies. The principal isolation study in progress during the year was concerned with the human macromolecular vasodilator "kallikrein." It is currently believed that kallikrein is a pancreatic product, present in plasma and in urine, that acts on a normal protein substrate (kallidinogen, a component of the α_2-globulin fraction) to yield a dialyzable polypeptide (kallidin) with profound vasodilator properties. The kallikrein is held in combination in the circulatory system by a protein inactivator and its vasodilator properties are exerted only when this complex dissociates to give free kallikrein; when this occurs the action is mediated through the polypeptide kallidin. The physiologic significance of these relationships is not known, but it is now clear that there are perhaps several kallikrein-like substances present in the human.

This problem is being studied (Dr. Pierce) in collaboration with Dr. S. J. Sarnoff and his colleagues. The chemical work is concerned with the problem of isolating kallikrein and its substrate kallidinogen, and ultimately kallidin, so that they may be defined as chemical individuals. A particular need is to isolate kallidin for study and to determine whether the several compounds of this type described in the literature are identical. The technical problems are quite difficult; the isolation of protein or polypeptide material in a high state of purity usually requires extensive fractionation, and in this case relatively small amounts of material are available. Several procedures have been studied for kallikrein or kallidinogen. The best results were obtained with a combination of XE-64 resin and DEAE cellulose. Hog pancreatic kallikrein (Padutin) was also studied, but the major part of the work has been with human materials. Relatively good fractions have been prepared, but these are not yet pure enough for characterization by chemical means, and the present work is concerned with getting still purer samples of kallikrein and kallidinogen.

The physiological experiments and work with hypotensive patients were carried out in Dr. Sarnoff's laboratory. A urine assay procedure was worked out and has been published.

The urine base project (Dr. Fish) was terminated in July. Seven bases of unknown structure remain from this work.

The alkaloids of Himantandra belgraveana were isolated in an attempt to throw some light on the "Kuru" disease of New Guinea. It was found (Miss Zaltzman) that himbacine was a physiologically active agent, producing convulsions ending in death, and that this action duplicated that observed for the crude plant extract, which was reportedly taken orally in New Guinea. There is an Australian chemical group working on this problem at the present time, and it is unlikely that a structural study of the alkaloid will be pursued in view of the Australian work.

Several human metabolites of unknown structure, present in instances of anemia and malabsorption, were studied in conjunction with Dr. C. E. Dalgliesh of the Postgraduate Medical School, London. The isolation work was carried only far enough to fix the identity of the substances as skatole metabolites, and further work was directed to examining the biological and chemical reactions involved in the formation of these materials.

A problem directed to the isolation of the glycolipids of human red cells was started recently (Dr. Sweeley, Dr. Moscatelli). These compounds are rather complex substances made up of sphingosine (or one of its relatives) and a long-chain fatty acid which often carries an α-hydroxy group, glucose or galactose, and usually neuraminic acid. A distinguishing feature of these compounds lies in the fatty acid part; acids such as cerebronic, nervonic and lignoceric are reported to be present, and it is believed that different tissues contain different acids. One of the problems lies in finding a way to deal with α-hydroxy acids, so that they may be studied by gas chromatographic techniques; another lies in developing more effective isolation methods for glycolipids than have been used previously. The present work is on isolation methods.

B. Chemical and Biological Reactions. Studies in this area involved both chemical and biological work dealing with biological transformations, with the aim of establishing the basic chemistry involved in each instance. Amine oxide studies were continued. Evidence was found pointing to the existence of a cellular amine oxide rearrangement reaction (Dr. Fish, Dr. Sweeley). The stereochemical aspects were studied through the preparation of both diastereoisomers of nicotine oxide (Dr. Sweeley); one underwent demethylation readily, the other did not. Microbial demethylation studies did not lead to a definitive answer on the ability of the oxide to replace the amine in a biological demethylation reaction; this may be due to the inability of the oxide to enter

particulate structures (the two previously observed biological demethylation reactions for the oxide were found to occur in the soluble portion of a cellular fraction).

Studies on the catalyst requirements and on the basic chemical mechanism of the reaction have been started in Australia by Dr. Cymerman Craig, and these will be continued in Bethesda. The effectiveness of the catalytic effect depends on the nature of the iron-coordination complex.

Studies on the metabolism of tryptamine (Dr. Fish) have been terminated. A new product found and characterized by chromatographic and electrophoretic methods was quite similar to N-formyltryptamine in its properties, but a definitive structural assignment was not made. The problem is currently under study by Dr. Jepson in Dr. Udenfriend's laboratory.

The oxidation (hydroxylation) of tryptophan derivatives was studied to gain additional chemical knowledge of the reactions of these compounds. It was found that the major product of hydroxylation (iron-ascorbic acid system) was not the 5-compound, but an isomer. This reaction is under detailed study for skatole. Oxidation at the 2- and 3-positions also occurs. The biological products are under study and comparison with synthetic products, with the aim of determining the nature of the major biological hydroxylation route. It is not the 5-pathway. Hydroxylation followed by conjugation, and ring-opening followed by hydroxylation and conjugation are the two chief reactions in the human and in the rat. o-Aminoacetophenone, a lipid-soluble aromatic amine, is one of the biological intermediates.

C. Structural Studies. The major work in this area has been concerned with the Amaryllis alkaloids (Dr. Wildman, Dr. Fales, Dr. Uyeo, Dr. Inubushi). The work of this group has established most of the current chemical (structural) knowledge of these compounds. The isolation studies have been relatively straightforward and most of the work has been devoted to establishing individual structures and structural relationships. A number of new structures were determined during the year, and an observation of considerable chemical interest was made in regard to cyclic strained lactams. Theoretical predictions had been made about the properties of certain specific lactam structures, but since none of these had ever been observed, the problem was one of discussion rather than experiment. A situation of this kind was found

during this work, and the compound had properties essentially as predicted. A second observation of biological interest concerned the physiological activity of these compounds. Several of them are analgesic agents of the same order of activity as morphine, but many are inactive. A proposal was made, on the basis of structural comparisons, that one (the active series) would have an "umbrella" or morphine-like structure, and the other would have quite a different stereochemical arrangement. This was disproved. It was found that all of the active compounds have the same general structural features, but that one series has an enantiomorphic relationship to the other, and that the "umbrella" structure is not present. The absolute configuration determines the activity, and all members of one group are active; members of the other group have no activity.

The lunasia and Ocotea problems (Dr. Goodwin) are largely completed. The Lunasia structural work involved quinolines and 2- and 4-quinolones; the Ocotea compounds are aporphines. Separate studies on NMR information, in relation to alkaloid studies, were undertaken. The results indicate that significant structural information may be obtained when possible structures and suitable models are compared.

The Ormosia problem (Dr. Lloyd) was carried through to publication, but terminated in July.

Tecoma and Cassia compounds were not studied during the year.

D. Methodology. Gas chromatographic methodology has been followed during the year, but very little direct experimental work was possible. Several liquid phases (Dr. Sweeley) were tested by Dr. Lipsky. An investigation of methods suitable for dealing with α-hydroxy acids is projected.

Laboratory of Cardiovascular Physiology

An attempt was made to examine in broader perspective the importance of the relationship generally known as Starling's Law of the Heart for the regulation of the intact circulation. The ventricular function curve concept was employed. More specifically, canine experiments were designed so as to ascertain whether nerve pathways could shift the baseline of the relationship between filling pressure and external ventricular stroke work. To do this, measurements were made of atrial, arterial and ventricular pressures while metering cardiac output. Pressure and flow were also measured in the independently perfused carotid arteries with exclusion of other arterial supply to the head. Heart rate was held constant in all experiments by atrial stimulation. Observations were made which demonstrated that stimulation of the isolated left stellate ganglion shifted the ventricular function curve to the left and that the extent of the curve shift is a function of the frequency and/or voltage of the applied stimulus. Stimulation of the distal cut end of either vagus produced the opposite effect, that is, a curve shift to the right. The order of magnitude of the changes observed was large. These experiments make it possible to put Starling's Law of the Heart in clearer perspective. That is, the central nervous system has available to it efferent pathways by means of which it can systematically manipulate the relationship between filling pressure and ventricular stroke work.

Further experiments were then done to ascertain whether these efferent pathways are exercised by changes in carotid sinus pressure. Stimulation of the carotid sinus nerves produced hemodynamic responses identical with those observed when withdrawing sympathetic stimulation or initiating vagal stimulation, that is, a shift of the ventricular function curve to the right. Elevation of the carotid sinus pressure had the same effect. Lowering the carotid pressure shifted the ventricular function curve to the left. The observed changes were marked. In summary these data demonstrated that the organism has available to it pathways by means of which the heart is caused to contract more forcefully at any given filling pressure when arterial pressure is low and, conversely, is caused to contract less forcefully at any given filling pressure when arterial pressure is high.

It was further observed that, with large changes in carotid pressure, total peripheral resistance changed one to two fold while changes in ventricular external work increased more than ten fold at

the same or even lower filling pressures. These data suggest that the dominant consequence of baroreceptor stimulation for circulatory regulation is the reflex effect on myocardial contractility rather than on peripheral vascular tone.

Investigations have been initiated to determine whether these nervous pathways are important in circulatory regulation in exercise.

While, as described above, attempts were made to relate the intimate performance characteristics of the heart to the overall blood flow requirements of the organism, experiments were continued of the type aimed at eliciting a more precise appreciation of cardiac energetics per se. Generally speaking these were of three types.

The first utilizes the moving of a lever by the myocardium in relation to a second fixed point which does not move. The change in distance between these two points is signaled to a recorder through a low-inertia, microtorque potentiometer. The recorded changes in "fiber length," when examined in relation to the pressure events of the cardiac cycle, by and large appear appropriate, e.g. fit our preconceived notions of what is occurring. The principal interest in this measurement is to be able to follow changes in diastolic fiber length, systolic fiber length, and rates of change in contraction and relaxation. With the other simultaneously recorded parameters it appears that we are able to examine for changes in end-diastolic distensibility under the influence of both cardiac sympathetic and vagal stimulation to ascertain whether any such changes are due either to the specific influence of the nerve stimulation or changes in the duration of diastole or both. Eventually it is hoped to examine more in detail the supposed relationship between end-diastolic fiber length and myocardial O_2 consumption.

The second type of experiment in this group resulted from an incidental observation that filling pressure rose and ventricular work fell when the site of electrical excitation was abruptly changed from atrium to ventricle. A systematic study was initiated to study this phenomenon. This consisted of the measurement of pressures in left and right atria, pulmonary artery and aorta and, in some experiments, also in the left ventricle. Total aortic flow (C.O. minus coronary flow) was also recorded and data obtained for the construction of ventricular function curves. It was observed that the change from an atrial to a ventricular site of excitation produced a pronounced shift of the ventricular function curve to the right, that is, less external work at any given

filling pressure. In many instances, this effect was such that there was more than 50% decreased in external work at the same filling pressure. It was also observed that myocardial oxygen consumption rose when going from atrial to ventricular stimulation which, since external work fell, resulted in a decrease in myocardial efficiency. One possible explanation for the observed phenomena being entertained is that the contraction of ventricular fibers is less synchronous when one excites what is essentially an ectopic focus than when the impulse is propagated normally. In support of this view are the observations by others that the electrical propagation of an impulse originating in the ventricle is slower than an impulse of atrial origin. We had a further strengthening of this interpretation from our observations that the upslope of ventricular pressure is less steep as is the curve showing the rate of fiber shortening as measured by the newly developed length lever. That is, if the impulse is less well coordinated and propagated less rapidly, the initial and last fibers to contract are doing so with the remainder of the ventricle acting as a flaccid aneurysm more than would be the case when the contraction is more synchronous.

An additional mechanism which appears to be involved in the observed results is the asynchronicity (relative to normal) of the sequence of atrial and ventricular contraction such that the ventricle is less abundantly filled when the atrium contracts against a closed mitral valve.

We can only guess at the significance of these findings for clinical heart disease. They would appear to furnish a more realistic basis for explaining the consequences of ventricular arrhythmias than has been previously available, especially when compounded by tachycardia. One is also impelled to wonder whether certain degrees of asynchronous myocardial contraction might contribute to the limited performance characteristics observed in clinical heart disease.

Following the incidental observation that arterial pressure rose when visceral vascular hypotension was induced a systematic inquiry was formulated. It was found that intravascular hypotension in the cat abdominal viscera supplied by coeliac, superior mesenteric and inferior mesenteric arteries produced prompt and substantial increases in the systemic arterial pressure and heart rate. In the majority of cases, this effect was seen even with intact carotid sinus and aortic arch baroreceptive systems but was accentuated after vagotomy and carotid sinus denervation. Intravascular pancreatic hypotension also produced elevations

in the systemic arterial pressure. These pressure rises were usually two thirds to three quarters as great as those observed during occlusion of the three major abdominal vessels. Intravascular hypertension, on the other hand, in the superior mesenteric artery caused a fall in the systemic arterial pressure. From nerve section experiments, it was concluded that the afferents of this abdominal baro-sensitive system were not carried in the vagus nerve, but rather in splanchnic afferent fibers. The reflex effects of this system were blocked by tetraethyl ammonium and hexamethonium chloride. The previous work of Gammon and Bronk, together with these data, combined to suggest that Pacinian corpuscles in the splanchnic area may play a substantial role in the regulation of arterial pressure in the cat.

The interest of this laboratory in the possibility that the highly potent vasodilator in human urine might reflect the operational significance of blood levels of the substance in question has been furthered by the addition of chemical personnel to the laboratory and the aiding of their efforts to chemically isolate the substances at hand by help from the previously developed method of bio-assay. It has been found that citrated human plasma when brought to a level of 30% acetone will yield a dilator substance of such potency that 0.5 cc of a 1:100 dilution of such a solution of human plasma will double or triple femoral arterial blood flow in the dog. 30% acetone in Tyrodes had little or no effect. SBTT (soybean trypsin inhibitor) known to inhibit callicrein also inhibited this reaction.

Earlier studies have shown that patients with orthostatic hypotension gave a decreased urinary excretion of a non-dialyzable vasodilator substance. Further studies have been conducted to determine the identity and physiological significance of this substance.

It had previously been noted that this non-dialyzable vasodilator had biochemical properties similar to callicrein, a hypotensive enzyme of endogenous origin. Correlation of a series of partially purified preparations from hog and bovine pancreas and from human urine for their vasodilator and hypotensive effect indicated that the biological activities were measuring one substance.

Callicrein is reported to exert its hypotensive effect by enzymatic action on callidinogen, present in normal plasma, to release a smaller molecule, presumably polypeptide, called callidin. Callidin can be differentiated from callicrein by its ability to contract guinea pig intestine,

and the callidinogen content of plasma or serum can be determined by addition of an excess of callicrein to the tissue bath. It was found that certain crude callicrein preparations from hog and bovine pancreas failed to cause contraction of the tissue when added to human plasma, although more highly purified preparations from bovine pancreas were satisfactory in this regard. All callicrein preparations from human urine when added to human plasma furnished maximal contraction of the tissue. Since the addition of human urinary callicrein to plasma, previously treated with crude hog pancreatic callicrein, still fails to release a substance capable of contracting the intestine, it is possible that this hog pancreatic callicrein had digested the callidinogen but contained an enzyme which immediately destroyed the polypeptide. Infusion of relatively large amounts of the hog pancreatic callicrein (300 Frey units) into dogs (2) caused a lowering of the blood pressure and a reduction of their callidinogen content of approximately 60% (sample taken three hours after infusion).

As an initial approach to this biological system of callicrein, callidinogen, callidin and their respective inhibitors, it was decided to attempt the isolation of callidinogen, the substrate for initial enzymatic activity of callicrein. However, heavy losses have been incurred in all purification procedures tried to date. It was found that out-dated citrated plasma was an excellent source for callidinogen. However, Cohn's fractions prepared by two manufacturers gave very poor yields of callidinogen (10%). Callidinogen was stable in citrated plasma for at least one month at pH 6.0. However, at more alkaline pH, the callidinogen content was markedly lowered in three to six days storage at 4°C. This was due to activation of one of the plasma proteinases, since heating the plasma for two hours at 56°C or the addition of crystalline soybean trypsin inhibitor (SBTI) prevented the destruction of the callidinogen. Dialysis has also been found to activate proteinases which digest callidinogen and are inhibitable with SBTI. Since both plasmin and blood callicrein are known to be capable of digesting callidinogen and both proteolytic activities may be inhibited with SBTI, it is possible that either one or both of these proteinases are activated by destruction or removal of their inhibitors.

Callicrein can now be prepared from human urine by direct-batch-wise adsorption on XE-64 resin. Callicrein prepared by this method is five times purer than callicrein prepared by adsorption on uranium acetate. Preliminary studies have been initiated on the best method for the initial extraction and precipitation of callicrein from human pancreas.

The immediately aforementioned experiments on the callicrein system and the previous experiments indicating the readiness with which arterial hypertension could be induced by stellate ganglion stimulation suggested the possibility that it might be worthwhile to study the output of urinary vasodilator substance during the hypertension so induced. Surprisingly, a marked diuresis occurred. The objectives of the experiment were then altered in order to examine more adequately the details of this interesting phenomenon. The data from nine complete experiments show that upon stimulation of the isolated stellate ganglion in the dog there occurred an immediate diuresis which appeared to be independent of arterial pressure, and that during the diuresis glomerular filtration rate usually showed little change and total urinary solute excretion increased. Following vagotomy, the diuresis was significantly reduced although the elevation of arterial pressure was at least the same or, more frequently, greater than that obtained before vagotomy. Left atrial pressure decreased during stellate stimulation both prior to and following vagotomy and in seven of the nine experiments the diuresis was a function of the fall in left atrial pressure. The data suggest that, although there may be receptors in the heart which upon adequate stimulation can modify urine flow as suggested by Gauer and co-workers, the adequate stimulus does not appear to be an increased left atrial pressure.

Laboratory of Kidney and Electrolyte Metabolism

Four major areas of research are being pursued in the Laboratory of Kidney and Electrolyte Metabolism. These include: 1) studies of the mechanisms of water and electrolyte transport in biological systems, 2) studies of the altered physiology in experimental heart failure in animals, 3) studies of the control of aldosterone excretion in dogs and 4) studies of a cardiotonic protein system in serum of hypertensive patients.

A theory relative to the mechanism of elaboration of both a concentrated and dilute urine has been published by Drs. Berliner, Levinsky, Davidson and Eden. In general it has been postulated that tubule urine becomes dilute by virtue of active transport of Na^+ from urine into renal interstitial tissue. If no vasopressin is secreted, the tubule membrane distal to the dilution site remains virtually impermeable to water so that the final urine remains less concentrated than plasma. If vasopressin is present, the membrane becomes permeable to water, water is abstracted by virtue of the increased osmotic pressure of the interstitial fluid (hypertonic in consequence of the transport of Na^+ out of the urine into the interstitial fluid alluded to above) and hypertonic urine is elaborated. Maintenance of the high interstitial osmotic pressure is provided by a vascular countercurrent system in the medullary area of the kidney. The presence of an osmotic gradient (increased osmotic pressure in the pertinent area of the kidney) has been confirmed by Levinsky, Davidson and Berliner. Direct experimental proof of the theory is now being attempted. The prediction that urine in the loop of Henle would be dilute under all circumstances is probably correct insofar as the ascending limb of the loop is concerned, but that urine concentration at the tip of the loop is not hypotonic has been shown by Gottschalk. In the light of this finding the theory requires modification and it has been tentatively proposed that only the ascending limb of Henle is impermeable to water.

In order to provide information concerning the solute and water movements involved in this mechanism, measurements of osmotic pressure, electrolyte content, and urea concentration of tubule urine in the loop and elsewhere are planned. Analysis of medullary blood is also to be attempted. Dr. Kennedy is in the process of perfecting the micropuncture technique for use in small animals: hamster, rat, and necturus. Drs. Jaenike and Bray in collaboration with Dr. Bowman of

the Laboratory of Technical Development are perfecting microanalytical techniques for measurement of Na^+ and K^+ and urea.

Drs. Levinsky and Berliner have examined the role of urea in the concentration process. They have established in both dog and man that acute increases in urea excretion permit the elaboration of urine of greater concentration than when urea intake and excretion are restricted. Presumably the addition of urea to both urine and interstitial fluid provides additional solute on both sides of the tubule membrane and thus the basis for an increase in osmolality in excess of that accomplished by the driving force of the high interstitial concentration of sodium alone.

Variations in medullary blood flow theoretically should modify urine concentration. A decrease in flow should magnify the effectiveness of the countercurrent system and increase the osmotic gradient. Drs. Eden, Jaenike, Bray and Berliner have inserted an electrode responsive to Na^+ concentration into dog renal medulla and have observed a rise in Na^+ concentration when blood flow is diminished by partially occluding the renal artery. A similar rise has been noted during ureteral obstruction. Further studies are in progress.

Alterations in urine composition following complete ureteral obstruction (stop-flow technique of Wilde et al.) are also being investigated by Drs. Jaenike, Bray and Berliner. This procedure permits an approximate estimate of the site of the changes in urine composition along the length of the renal tubule during stopped flow. Pelvic dead space is being minimized by insertion of an inert oil prior to stoppage of flow. The influence of vasopressin on urine osmolality and urine composition during the period of stopped flow is under investigation. The results are still in a preliminary stage.

Drs. Levinsky and Berliner have found that both the ureteral and bladder membrane are permeable to water, electrolytes and urea. This is of considerable interest since diffusion of these substances during periods of low urine flow may significantly alter the composition of urine. Data relating to the effect of urine flow on urea excretion require reinterpretation in the light of this observation. Alterations in urea clearance initially interpreted as being conditioned by transtubular diffusion of urea may be secondary to diffusion of urea from bladder urine during periods of stasis. This aspect of the problem is now being investigated by Drs. Jaenike and Bray in the dog.

Drs. Orloff and Burg have been examining the effects of various agents on electrolyte excretion in the chicken. The chicken possesses a renal portal circulation so that substances injected into the leg vein perfuse the peritubular area of the kidney on that side prior to entering the general circulation. By comparing the effects of a test substance on urine composition of the injected side with that of the contralateral control, it is possible to determine both those alterations due to direct tubular effects of the substance on transport and the probable site of action of the test substance. Using this technique it has been shown 1) that K^+ is secreted in the chicken, 2) that it is transported by a system which is capable of saturation, 3) that it is subject to competitive inhibition by H^+ ions. The demonstration of a tubular maximum for K^+ is the first such demonstration for a strong electrolyte in any species. Studies by Drs. Orloff and Davidson are in press.

Drs. Orloff and Burg have also studied the effect of strophanthidin, a cardiac aglycone, on tubular transport of electrolytes. They have shown that the drug is a powerful diuretic, increasing sodium excretion markedly. Changes in both K^+ and H^+ excretion are consistent with the hypothesis that the aglycone interferes with the tubular transport system by which K^+ and H^+ ions are secreted into urine in exchange for reabsorbed Na^+. A uniform depression in the secretion of the anion, paraminohippurate, has also been observed. The electrolyte effects are thought to be analogous to those observed in the red cell, skeletal muscle, cardiac muscle, and kidney slices (see below). In collaboration with Dr. S. Hajdu, Drs. Orloff and Burg have demonstrated 1) that the strophanthidin is secreted by the tubule cells, and 2) that it probably acts on the contraluminal border of the tubule cell.

No effects of salt-active adrenal steroids including aldosterone have been observed in the chicken kidney in acute studies. Vasopressin on the other hand diminishes water excretion and promotes the elaboration of a hypertonic urine. Vasopressin, an easily filterable polypeptide, may act on the contraluminal border of the tubule cell since a unilateral effect of small doses has been observed.

The mechanism of ammonia excretion continues to be a primary interest of this laboratory. "Urinary adaptation" (increased ammonia excretion at the same urine pH) in the rat is due to an increase in the activity of glutaminase, the enzyme which accelerates the deamidation of glutamine. This is clearly not the basis for adaptation in the dog. No difference in the activities of any of the renal enzymes known to be

capable of providing ammonia from amino acids were observed when either renal cortical slices or homogenates of renal tissue from acidotic and alkalotic dogs were examined (Drs. Rector and Orloff). It has not been possible to determine whether the observed effects of acidosis are due to an increase in membrane permeability and influx of precursor amino acids. Of interest, however, is the observation that the pattern of ammonia excretion in "adapted" dogs following the infusion of either glutamine or alanine is similar to that of alkalotic or normal animals. No acceleration of conversion of the amino acid to ammonia was observed. In the rat the urinary response to alanine was unlike that of the dog. However, definitive conclusions have not been arrived at. Further studies are in progress.

Dr. Cotlove has developed a precise, reproducible, electrometric method for measurement of true tissue chloride. He is now engaged in a systematic study of the distribution of ions in various tissues. The results are still in a preliminary form.

Drs. Burg and Orloff have examined the kinetics of Na^+ and K^+ exchange in slices of rabbit renal cortex. They have performed a large series of studies in an attempt to develop a reproducible and accurate method for measuring electrolyte fluxes in single slices of rabbit renal cortex. The method finally adopted is theoretically superior to others in the literature and should provide much useful information. They have been able to estimate K^+ influx by measuring the uptake of K^{42} by a single slice in a well-scintillation counter from medium of constant specific activity. Efflux has been determined by the reverse process, the washout of K^{42} from a single slice into medium free of isotope. Strophanthidin has been shown to interfere with K^{42} influx without appreciably affecting K^{42} efflux. Studies of the effect of other drugs are in progress. It has also been shown that the influx of potassium increases with increasing medium potassium until saturation is achieved. Studies on sodium flux have been unsatisfactory to date. However, the negative results are of interest since it is apparent that conclusions relative to absence of linked Na-K exchange in cortical slices based on similar findings of others need not be valid.

Drs. Burg and Orloff have examined the effects of strophanthidin on the uptake of PAH, Na^+ and K^+ transport, and O_2 consumption in renal cortical slices. They have shown that the drug decreases the K^+ content of kidney slices and increases that of Na^+ without appreciably affecting O_2 consumption. An observed interference with paraminohippurate accumulation has been shown to be secondary to the effect on K^+ influx.

Drs. Hoffman and Tosteson have studied Na+ and K+ transport in human red cell ghosts and in sheep red cells. Studies in red cell ghost have been directed at elucidating the role of metabolic factors in ion transport and in examining the effect of Mg++ and Ca++ and chelating agents on K+ accumulation. It has been shown that the addition of inosine stimulates active K+ transport which is inhibited by strophanthidin. No differential metabolic effect of strophanthidin has been noted in this tissue as in others which have been studied. ATP has also been shown to act as a source of energy for active K transport in ghosts. This, too, is strophanthidin sensitive in that the rise in flux can be interfered with by the drug.

The magnitude of K+ influx or the initial K+ content of ghosts provides an inverse measure of the structural integrity of the plasma membrane. This observation therefore provides a method for the assay of compounds which affect membrane monovalent cation permeability. Cells hemolyzed in Na2ATP yield ghosts of low K+ content (increased permeability) whereas those hemolyzed in MgATP do not. Further studies using MgEDTA, etc. indicated that normal monovalent cation permeability probably requires the presence of a divalent cation (Mg++) to stabilize the internal molecular arrangement of the membrane.

Some individual sheep have red cells with high potassium and low Na content (HK) while others have cells with low K and high Na (LK). The LK gene is a Mendelian dominant. Studies of Na+ and K+ transport in both cell populations by Drs. Hoffman and Tosteson have demonstrated significant differences in the active and passive components of electrolyte transport in these groups. It appears that a single gene controls both the magnitude of active transport and resistance to passive diffusion of Na+ and K+ in sheep red cells.

Drs. Hajdu and Leonard are currently investigating the physiological properties of a cardiotonic protein system found in increased quantities in blood of hypertensive individuals. They had previously reported in detail on the isolation and purification of this system which affects frog heart contractility in a manner analogous to cardiac glycosides. The results of the present studies are still in a preliminary form.

The administration of KCl to abolish the clinical toxicity of cardiac glycosides is established medical practice. Drs. Leonard and Hajdu have provided evidence that the effect is not secondary to interference with the

action of the glycoside since in the animal at least, KCl does not abolish the positive inotropic effect of strophanthidin. These results are of some interest since other actions of strophanthidin are interfered with by K+. Thus Drs. Burg and Orloff were able to overcome the inhibitory effect of the drug on PAH accumulation of kidney slices by the addition of large amounts of K+ to the incubation mixture.

Drs. Davis and Yankopoulos have been studying the mechanism of increased aldosterone secretion in secondary hyperaldosteronism. In association with Drs. Kliman and Peterson of NIAMD they have shown that aldosterone excretion is markedly augmented by constriction of the thoracic vena cava in dogs. This was not inhibited by prior or concurrent expansion of the plasma volume, providing evidence that the fall in total plasma volume following venal caval constriction is not a necessary requirement for augmented aldosterone output. Aldosterone excretion has been shown to be in some way dependent on intact pituitary function. No clearcut evidence indicative of a role of either the diencephalon or hypothalamus was obtained.

In an effort to determine the role of the vagus, carotid and aortic depressor nerves on aldosterone secretion a number of studies were undertaken in collaboration with Dr. John Holman. To date all results have been negative. They have shown, however, that desoxycorticosterone output in adrenal vein blood may be increased in dogs with thoracic caval constriction.

Using cross-circulation experiments, Drs. Davis and Yankopoulos have obtained evidence that an unknown humoral agent is responsible for the stimulation of aldosterone excretion in dogs with experimental ascites. This is an extremely important observation. No information as to either the source of the humoral agent or the stimulus necessary for its secretion is as yet available. Studies aimed at providing this information have been instituted.

Drs. Davis and Yankopoulos have reexamined the physiological changes in experimental heart failure in dogs with tricuspid insufficiency and pulmonic stenosis. Although ascites and increased pressure in the right auricle were observed, there was marked variability in the degree of Na+ retention. No evidence of left ventricular failure was obtained.

Drs. Yankopoulos and Davis have continued their studies in the characterization of the contractile proteins of cardiac muscle from

animals with experimental heart failure. They have compared actomyosin from normal and failing hearts. No difference in the yield of actomyosin, its sedimentation pattern, viscosity or response to ATP has been noted. Studies on Myosin A are continuing. An important preliminary finding is the fact that the molecular weight of Myosin A from cardiac muscle appears to be the same as that from skeletal muscle. This is contrary to other reports in the literature. No clear-cut differences in the isometric tension patterns of glycerol extracted fibres from the two groups of hearts have been observed. Studies on water and Na^+ content of cardiac muscle from hypophysectomized animals are in progress.

Laboratory of Technical Development

The Laboratory's development of new and more sensitive methods and instruments is continuing along several lines. The development of methods for analysis of materials obtained by micropuncture techniques has shown some promise in the form of a method of analysis of the alkali metals by a rather simple, rapid method. This method utilizes the plasma glow of a radio frequency discharge in helium at atmospheric pressure to volatilize and excite the alkali metals from a platinum wire. The emission so produced is separated from the helium lines by means of a monochromator and measured by a photomultiplier and integrating galvanometer. Samples are deposited directly on the platinum wire from the micro-pipette and to date it appears that 10^{-11} moles of sodium can be estimated with an accuracy of 5%. Much greater sensitivity appears attainable but this method has only recently been subjected to intensive study. It appears to have advantage over previous approaches.

Freezing point determinations on micropuncture samples have been made by observation of the time of thawing. Adequate measurements can be made using samples in the range of several thousandths of a cubic millimeter, but the method still requires further evaluation.

The development of a new technique of extreme sensitivity for gas chromatographic analysis has been completed and several modifications of existing chromatographic techniques have been made. The development of the high sensitivity detector has made it possible to do an analysis on a few micrograms of fatty acid methyl esters so that methods for handling microgram samples had to be developed. Successful sample introduction syatems capable of accurately and reproducibly injecting a few micrograms of materials into heated entries to the column have been developed and a deacription published.

During the summer the nuclear magnetic resonance flow meter project was reactivated and a series of tests indicated that this technique could definitely be used to measure flow in a reproducible fashion. The necessity for additional electronic control became evident and a more versatile apparatus now under construction is nearing completion. The construction of this equipment is being made possible by our acquisition of Mr. Vsevolod Kudravcev's services. It is hoped that this apparatus will not only allow us to evaluate the application of nuclear magnetic resonance to flow measurement but will also provide working equipment

to test the sensitivity of broad line resonance methods as an analytical technique. The advantage of this technique lies in its being a non-destructive method of analysis as opposed to its having a high sensitivity method.

An electronic curve analyzer capable of synthesizing a composite curve of several pure functions has been completed. The composite function fitted to the sample curve is presumed to indicate the individual functions contained in the complex curve. The application of this apparatus to the analysis of mixed Gaussien distributions is expected to facilitate the analysis of many types of biological data normally available in this form. The preparation of pure samples for testing the apparatus on an optical problem involving the resolution of overlapping absorption curves is in progress.

Some refinements in the Fourier analysis apparatus have been made in the form of eliminating the need for a panoramic analyzer to confirm the attainment of the null point. A square law voltmeter has been shown to be a satisfactory null detector.

The sonic valve catheter tip transducer has been shown to be feasible but technical problems in construction of the tip and adequate sound transfer down the catheter still remain to be solved. A sonic interferometer utilizing some refined electronic techniques to increase the sensitivity of these methods has shown some promise of becoming a non-destructive bulk property device, which would allow predictable behavior as a gas chromatography detector.

The production of free hydroxyl radicals by ultrasonic cavitation has been reviewed and compared to the production of free radicals by ionizing radiation.

Instrumentation for analysis of fluorescent and phosphorescent materials has been continued along the lines of setting up a method for storing and later analyzing the emitted phosphorescent spectrum produced by a high intensity short duration light source. A photoconductive zinc oxide surface is electrified and following exposure to the flash spectrum the surface is "read out" by means of a vibrating reed probe or measured as the current produced by discharge of the paper by a moving beam of light. Both "read out" systems have been successful but the actual testing with phosphorescent emission has not

yet been done. Excitation of low probability triplet states by high intensity is presumed to provide structure information not otherwise obtainable. The search for lower energy fluorescence and phosphorescence in the near infrared may also be facilitated by the paper storage system as it becomes a storage-integration system.

A system for finding chromatographic "spots" (aldosterone) and other steroids utilizing the phosphorescence of the paper and material after cooling in liquid air. The phosphorescence of the paper was remarkably free from the influence by the steroids tested. The system should be tested further. The aldosterone was finally determined quantitatively by the measurement of the fluorescence observed through the paper in an improvised apparatus set up for Dr. Mill's use.

The development of prosthetic valves is continuing with Dr. N. Braunwald who has been putting the silastic tricuspid valve in the descending aorta of dogs with considerable success. Survivals have already exceeded a month in two cases and the strength of the valve has been much improved by attention to details in the molding process which is now done in our own press by Mr. Alexander McInnes. Dr. McMillan in St. Thomas' Hospital, in London, is collaborating in this work by testing our valves in his testing apparatus.

The blood oxygenator problem is continuing in the form of Dr. Ross' work, confirming the calculations by Dr. Booth that blood film thickness has to be in the range of 4 microns to realize the full effectiveness of polyethylene, and design for apparatus to attain this end is under consideration. An attempt to increase the effective transport of oxygen across the membrane by ultrasonic agitation was not successful with one frequency in a few relatively crude experiments.

Glass electrodes as small as 50 microns in diameter and about 500 microns long have been prepared which show a response of 58 millivolts per pH unit. Electrodes of similar size have been prepared which are sensitive to sodium and which show about 58 millivolts change for a 10 fold change in sodium concentration. These electrodes have been used in studies of kidney function by direct insertion into dog kidneys in vivo.

Investigation of the physical problems of freezing and drying has been continued. The drying rates of a number of materials including serum albumin, ovalbumin, blood plasma, and guinea pig

liver have been measured and used to compute the specific surface per gram dry weight of the frozen material. The efficiency of various coolants for rapid freezing has been measured. It has been found that to a good approximation of heat transfer at the surface is given by $H(T_s - T_f)$, where T_s is the surface temperature, T_f is the temperature of the coolant, and H, which is nearly constant, varies from 0.2 cals/cm^2 sec. deg. for liquid propane to .02 cals/cm^2 sec. deg. for liquid nitrogen. If H is known for a coolant it is possible to develop an exact theory for the k cooling of a solid of regular shape without phase change and an approximate theory for cooling with phase change. By application of the theory to experimental cooling curves it can be determined whether a phase change has occurred. Such an analysis of cooling curves of 10% gelatin blocks has indicated that for cubes less than 1 mm on edge, cooled in liquid propane there is only partial freezing of the water. Work has continued on the preparation of specimens for electron microscopy by freezing and drying. Here, in order to get a suitably uniform protoplasmic materia as a test object, colonies of both the amoeba Chaos Chaos and of paramecia have been started.

A study has been undertaken of the theory of transport in linear biological systems, typified by steady state tracer studies, and the application of this theory to specific problems in the kinetics of fatty acid metabolism. The basic theory relates fluxes in a system by an integral equation of the type: $\gamma_b(t) = \int_0^t \gamma_a(\omega)\, w(t-\omega)\, d\omega$, where w(t) is a transport function characteristic of the system. In multiflux problems, the fluxes are related by a system of such integral equations. General matrix methods for the solution of systems of this kind have been developed. This theory has been shown to give the well-known solutions for systems which consist of several uniformly mixed compartments, and has been applied to general problems of data analysis and model construction in systems with incomplete information. In collaboration with Dr. Donald Fredrickson, the theory has been applied to the analysis of tracer data on fatty acid metabolism, with the primary objective of comparing the metabolic pathways of unesterified fatty acid (UFA) and chylomicron triglyceride fatty acid (TGFA). Some initial hand computations have been carried out and the problem has been partially programmed for an electronic computer.

In addition to the regular projects, the Laboratory has provided space, collaboration and consultation with the Clinical and Professional Education Group, headed by Dr. Murray Brown, to evaluate the utility of color television techniques in biological research.

Also in addition to the research program of the Laboratory, special services not otherwise available have been provided to other laboratories of NHI.

Consultations on special problems in mathematics, electronics and mechanics have been provided. Tools and materials, electronic components and shop facilities have been supplied whenever regular service facilities proved inadequate for the exigency of the situation.

Members of the Laboratory have been active in stimulating the emergence of a group of individuals having interest and knowledge in the application of physical, electronic and other technological advances of industry to the problems of medical research and practice.

Laboratory of Chemical Pharmacology

Biochemistry of Function

Much of the current work in this laboratory is related to our philosophy that the gap between physiology and pharmacology on one side and biochemistry on the other stems largely from the assumption that specific organic functions can be explained in terms of the "universal" reactions of intermediary metabolism. Investigations in this laboratory are based on the belief that specialized functions are closely linked to unique substrates, as, for example, peripheral nerve conduction is closely associated with the substrates acetylcholine and noradrenaline (NE).

Role of Norepinephrine and Serotonin in Brain

Most drugs which influence the peripheral nervous system act at junctions where nerve impulses are transmitted by acetylcholine or NE. By analogy drugs should affect the central nervous system (CNS) by modifying chemical transmission at synaptic junctions. Evidence for this is difficult to obtain because CNS structures are inaccessible and because acetylcholine has been the only established central neurohormone. The discovery of the uneven distribution in brain of the intensely active amines, NE and serotonin (HT), makes it reasonable to assume that these substances have specialized functions in areas where they are found.

The conceptions of W. R. Hess have been used as a framework for bringing together biochemical, physiological and pharmacological aspects of brain function. Hess indicated that two opposing systems, ergotropic and trophotropic, integrate autonomic with somatomotor and psychic functions in brain stem. The ergotropic division integrates sympathetic with somatomotor activities to produce the behavioral patterns of positive action. The overall effects of ergotropic predominance are arousal, increased sympathetic activity, enhanced muscle tone and an activated psychic state. In contrast the trophotropic division integrates parasympathetic with somatomotor activities to produce behavioral patterns, recuperative in nature. The overall effects of trophotropic predominance are drowsiness and sleep, increased parasympathetic activity, decreased skeletal tone and activity, and lowered response to external

stimuli. Both systems are in continuous opposition, the resulting level of activity ranging from trophotropic predominance (sleep or apathy) to ergotropic predominance (excitement or mania).

It has been assumed that the two systems require different synaptic transmitting agents. Last year preliminary evidence was given that NE was the ergotropic hormone and HT the trophotropic. Results now indicate more definitively that the ergotropic division is an adrenergic system. Thus dihydroxyphenylalanine (DOPA), a NE precursor, penetrates brain and evokes a syndrome identical to ergotropic predominance. DOPA elicits EEG arousal, indicating stimulation of the reticular activating system. Thus, it may be proposed that the reticular activating system of the neurophysiologist, the ergotropic system of Hess, and the central adrenergic system of the pharmacologist are one and the same.

Central Adrenergic Agents

When analogs of NE are lipid-soluble, they penetrate the brain and may simulate NE at central adrenergic receptors. Examples are ibogaine, amphetamine, ephedrine, desoxyephedrine, Meratran, Ritalin, tetrahydro-β-naphthylamine, mescaline and LSD. (LSD is no longer considered to act in brain by blocking HT but by mimicking NE.) Cocaine, though not a NE analog, is also an ergotropic agent. These compounds produce excitement, increase psychomotor activity, and enhance central sympathetic output. Some of the compounds have a dual action and also mimic NE at peripheral sites, e.g., amphetamine and ephedrine increase blood pressure and heart rate by direct peripheral as well as by central action. In contrast, LSD and cocaine act mainly centrally. Of particular interest is the observation that barbiturates suppress the effects of ergotropic agents. Thus, many erroneous conclusions concerning actions of LSD, mescaline and other drugs on the cardiovascular system may be drawn from studies on anesthetized animals.

Central Adrenergic Blocking Agents

Chlorpromazine, known primarily for its hypotensive and sedative activities, also produces a multiplicity of effects almost exactly opposite to those of ergotropic predominance; it is considered to suppress the ergotropic system by blocking the action of brain NE. As expected, chlorpromazine counteracts the pharmacologic effects and the arousal EEG induced by DOPA, amphetamine, cocaine and LSD.

Chlorpromazine and other phenothiazines have been conclusively shown to decrease sympathetic activity by a central action and have little or no direct peripheral action. It is pertinent that the pressor effects of cocaine and chlorpromazine are competitive, suggesting that they compete for the same receptors.

Compounds That Release HT and NE

Compounds like reserpine elicit sedation, hypotension and a variety of other effects like those of chlorpromazine. It is believed that they do not act per se but by liberating amines through an action on the mechanisms that concentrate the amines in body tissues. Reserpine appears to stimulate the trophotropic system through the action of free HT. In considering reserpine's mode of action it must be kept in mind that it impairs the storage of NE and HT but not their formation. When stores are depleted, synthesis of the free amines continues. If formed slowly, the amine may be completely metabolized by monoamine oxidase (MAO) and the level at receptors be too low to elicit a response. Under these conditions reserpine may be considered to have depleted synaptic junction of hormone. Contrastingly, if the amine is formed rapidly, the level at receptors may be sufficient to produce a persistent response.

Studies on the turnover of brain HT indicate that it has a rapid turnover, 50 per cent in 10 to 15 minutes, compared to several hours for NE. Thus, after reserpine administration, brain HT may be formed rapidly enough to produce a trophotropic response. These results are consistent with the thesis that free HT is the important factor in central reserpine action.

The general opinion that the sedative and hypotensive actions of reserpine have a common central mechanism must now be modified. In low doses, reserpine depletes peripheral NE without affecting brain HT or NE and without producing sedation. As a result, functional sympathetic denervation is produced. A complete separation of peripheral and central effects is seen with SU 3118 (Ciba), a semi-synthetic analog of reserpine. In small daily doses, this drug depletes peripheral NE without lowering brain amines. Animals are not sedated but show bradycardia, hypotension, diminished response to carotid occlusion and other effects of chemical sympathectomy.

NE depletion, however, does not account for central effects of reserpine. This has been shown with compounds that affect NE binding in preference to that of HT. For example, small doses of SU 5171 (Ciba), a reserpine analog, depletes brain of NE but not of HT, and animals are not sedated. However, higher doses lower brain HT and also produce sedation.

Pharmacologic evidence that reserpine does not act centrally by NE depletion was obtained from observations that, unlike chlorpromazine, the drug does not decrease central sympathetic output, but increases parasympathetic output. Thus reserpine elicits miosis, lacrimation, nasal secretion and enhancement of light reflex by central parasympathetic action. The decreased sympathetic activity, e.g., decrease in blood pressure and relaxation of the nictitating membrane, is explained by peripheral depletion of NE.

A dramatic difference between reserpine and chlorpromazine is that reserpine elicits tonic closure of eyelids and extreme miosis as in normal sleep, while chlorpromazine produces slight miosis and relaxation of eyelids (ptosis). This accords with the notion that reserpine may cause sedation by stimulation of a "sleep" center and chlorpromazine by suppression of an arousal mechanism.

Monoamine Oxidase Inhibitors

A number of compounds that inhibit MAO are used in the treatment of depressed mental states, hypertension and angina pectoris. Iproniazid, JB 516, JB 835 and JB 807, all of which elevate brain levels of HT and, in certain species, NE, have been studied in this laboratory. JB 516 and JB 835 are extraordinarily potent agents -- about 50 times more so than iproniazid. Evidence suggests that the central excitation produced by these compounds is related temporally to the rise in brain NE rather than brain HT. Furthermore MAO inhibitors elevate brain HT but not NE in cats and dogs. These animals are not excited by the drugs.

The elevation in brain NE produced by the inhibitors indicates that MAO fulfills an important role in the biologic inactivation of this amine. O-methyltransferase does not seem to be involved in the inactivation of brain NE. In fact, direct evidence indicates that brain NE is metabolized almost entirely by MAO. There is some evidence that the

main role of MAO is not the metabolism of NE released at nerve endings but the regulation of the amounts of NE and HT within the neuron so that they do not continuously spill onto receptor sites.

Anticonvulsant Properties of Monoamine Oxidase Inhibitors

MAO inhibitors elevate brain amines and block electroshock convulsions in rats and metrazol convulsions in mice, in contrast to reserpine which lowers the brain amines and enhances the seizures. Strong evidence suggests that the effects of reserpine and of MAO inhibitors are mediated through the change in brain amines: 1) After the administration of a MAO inhibitor the anticonvulsant effect is closely related to the rise in amine levels; 2) after reserpine the enhancement of convulsions is related to the decline in amine levels; 3) release of amines by reserpine, before treatment with iproniazid, elicits the enhancement effect of reserpine; 4) iproniazid followed by giving reserpine elicits only the anticonvulsant effect of the MAO inhibitor. However, evidence that either NE or HT is the important factor is equivocal; an unknown amine released by reserpine and the metabolism of which is blocked by MAO inhibitors may be involved.

Since the blockade of MAO represents a new type of mechanism in the suppression of experimental convulsions, a clinical trial of the drugs is in progress in collaboration with Dr. Shy.

Role of Catecholamines in Fat Deposition

NE or epinephrine in doses of 0.5 to 1 mg per kg, infused over a period of several hours, induce a reversible fatty infiltration in heart and other tissues, associated with a reversible hypersensitivity of heart to epinephrine-induced arrhythmias. The lipid changes are blocked by dibenzyline. These studies have been extended to the effects of CCl_4, alcohol and ethionine. In single doses these substances cause a triglyceride infiltration in rat livers, which is blocked by the irreversible adrenergic blocking agents, dibenzyline, dibenamine and the reversible agent, ergotamine.

These studies are of potential importance since 1) catecholamines may be involved in fat transport; 2) the primary step in drug liver damage may involve interference with a catecholamine mechanism that regulates

fat transport. The possibility exists that adrenergic blockers may be used to protect against the liver toxicity of alcohol.

Role of Histamine

Studies on the role of histamine are hampered by laborious bioassay procedures. A chemical procedure has been developed which involves condensation of histamine with a dialdehyde to form a fluorescent product. The method permits the estimation of 0.01 γ of the base, a sensitivity approaching that of bioassay.

Studies with this method, especially on brain, should expedite knowledge on the function of histamine, its biosynthesis and metabolism and the effects of drugs which may act by depleting the substance or interfering with its metabolism.

Cardiotonic Substances in Tissues

Last year's report indicated that the digitalis-like material in tissues was in part lysolecithin. Other digitalis-like substances have been shown to include unsaturated acidic lipids containing 18 to 20 carbon atoms and containing a β-diketone group and an α-β unsaturated acid of about 26 carbons. These compounds may be involved in membrane function.

A Fluorescent Substance in Nasal Mucosa

Butanol extracts of dog nasal mucosa contain a fluorophor which behaves as a single compound on paper chromatograms and countercurrent distribution. The compound is neutral and has no biologic activity on smooth muscle preparations, and it seems to be present only in nasal mucosa. If the substance is present in other animals, and if it is not due to bacterial contamination, attempts will be made to isolate it and ascertain its function.

Membrane Permeability

Passage of Foreign Compounds Across Body Membranes

Body membranes behave as lipid barriers to foreign compounds, i.e., the rate of passage of a drug depends on its dissociation constant.

the lipid solubility of the unionized moiety, and the pH at the absorbing site. Several studies have been concerned with further elaboration of this concept.

Intestine

The steady state distribution ratios of drugs between plasma and small intestine are predictable if it is assumed 1) that the intestinal membrane secretes hydrogen ions that maintain a zone at its surface at a "virtual" pH of 5.3, and 2) that foreign compounds are absorbed largely in unionized form and at rates proportional to lipid solubility. Similar results were obtained for the colon using a "virtual" pH of about 6.5. These results explain the poor absorption of many water soluble drugs.

Blood-Brain Barrier

Foreign compounds penetrate into the CNS almost exactly as predicted on the basis of their physicochemical properties and the assumptions that 1) only the unionized form crosses and 2) this moiety enters at a rate proportional to lipid solubility.

Preliminary results indicate that barbital passes slowly from bloodstream into brain, thence into CSF. This suggests that the choroid plexus is not important in the passage of drugs into the CSF.

Certain foreign compounds have been found to penetrate rapidly the pituitary gland, pineal gland and the area postrema, whereas they slowly penetrate the brain as a whole. This finding not only suggests that these areas are not part of the brain but that like the pituitary they may also have secretory function. The observation with the pituitary may explain its sensitivity to epinephrine, despite the poor penetration of the amine into brain.

Bile-Blood Barrier

Previous work disclosed that inulin and sucrose, large lipid-insoluble substances, appear in bile in significant amounts, indicating porosity at some locus in the hepato-biliary system. Further studies have included a smaller molecule, mannitol, and indicate that small molecules pass the "blood-bile barrier" more readily than large molecules. Mannitol occupies a volume of distribution approximating

total water of liver; inulin and sucrose occupy a smaller space but still one that is probably greater than extracellular fluid. Preliminary results suggest that lipid-soluble foreign compounds do not appear in bile in appreciable amounts but that lipid-insoluble metabolites and quaternary compounds may appear in high concentration.

Passage of Normally Occurring Substrates Across Body Membranes

The view of a lipoidal boundary between intestine and plasma permits inferences as to whether a substance is absorbed passively or by a specialized process. The intestinal absorption of thymine and its nucleoside thymidine involves a transport mechanism. Thus, the degree of their absorption decreases with increasing concentration. Uracil depresses the absorption of thymine (5-methyluracil) by competing for the mechanism. Preliminary results suggest that a number of purine and pyrimidine derivatives compete for the same transport mechanism. In contrast, high concentrations of D-glucose or L-histidine do not inhibit the transport of thymine.

Drug Metabolism

The metabolism of drugs is discussed in terms of three categories of substances:

(1) Substances metabolized by relatively specific enzymes involved in intermediary metabolism

This laboratory has extended its studies of drug metabolism to the so-called antimetabolites, substances structurally so similar to normal substrates that they become involved in the "universal" enzymes of intermediary metabolism. These compounds are used as anti-cancer agents and their metabolism and mechanism of action are closely intertwined. 6-Chlorpurine (6-CLP) has been found to be a competitive inhibitor of purine and hypoxanthine oxidation by xanthine oxidase. It is converted to 6-chlorouric acid (6-CLU), a heretofore unknown compound which has been crystallized and characterized. Two other products are also formed in vivo including uric acid. 6-CLP also enters into synthetic reactions and is incorporated into nucleic acid. Experiments under way will ascertain if any 6-CLP metabolites account for its anti-cancer effects.

(2) Substances acted on by relatively non-specific enzymes involved in intermediary metabolism

Alcohol and aldehyde dehydrogenases are examples of extremely non-specific enzymes. The normal role of aldehyde dehydrogenase but not of alcohol dehydrogenase is known. Alcohol dehydrogenase is involved in the metabolism of drugs from which an alcohol is formed as the first step in their biotransformation, e.g., barbiturates. Studies are being undertaken to investigate the possibility that variation in human tolerance to alcohol may be based on differences in alcohol dehydrogenase activity. Preparatory to investigating the biologic variation of the enzyme activity in a large population, the substrate specificity of the enzyme is being studied. Surprisingly, higher alcohols have a greater affinity for the enzyme than ethanol. It is possible that other alcohols may prove to inhibit ethanol oxidation and thus provide an interesting experimental tool.

(3) Substances acted on by extremely non-specific enzymes not involved in intermediary metabolism

Most drug enzymes fall into this category. Further studies on the oxidative enzymes in liver microsomes have been made.

(a) The enzyme that oxidizes chlorpromazine and other sulfur compounds to the corresponding sulfoxide derivative has been studied. It too requires TPNH and O_2.

(b) The microsomal enzyme that oxidizes hydrocarbon sidechains to alcohols also requires TPNH and O_2. This is an important enzyme in maintaining body homeostasis since food contains many terpenes which cannot be excreted unless oxidized.

(c) The key reaction in the metabolism of nicotine occurs in microsomes and requires TPNH and O_2. It involves oxidation of the carbon atom next to the N in the sidechain. The next step to study presumably involves ring splitting, a reaction not yet elucidated for drugs.

(d) Monoamine oxidase inhibitors all seem to antagonize a number of microsonal enzymes. This peculiar action is unrelated to inhibition of MAO; in fact it is reversible and disappears by the time that MAO is completely blocked.

(e) A finding of great potential importance in connection with induced enzyme synthesis is that barbital and chloretone, which enhance the pathway of glucose metabolism that results in increased ascorbic acid formation in rats, also increase activity of the liver enzyme which demethylates aminoazo dyes.

(f) Two important steps have been taken in the elucidation of the nature of the microsomal enzymes: (1) Like microsomes, a number of model systems produce active hydroxyl groups and dealkylate alkylamines, form sulfoxides, hydroxylate aromatic rings, and split ethers. (2) Evidence has been found that TPNH and O_2 form an active hydroxyl-enzyme complex which acts as a hydroxyl donor. This donor is used up in direct proportion to amount of drug that is metabolized.

Biochemical Evolution

The exceptional non-specificity of microsomal enzymes, their inhibition by SKF 525-A and iproniazid, and their enhancement by a number of polycyclic hydrocarbon compounds suggest that many drug enzymes are not part of intermediary metabolism. The inability of the kidney to excrete lipid-soluble substances suggests that the oxidative enzymes were developed as special, non-specific mechanisms for making fat-soluble foreign substances ingested in food less lipid-soluble. On this basis drugs are models of lipid-soluble foreign compounds ingested by animals over the ages.

Oxidative drug enzymes are absent in fish and aquatic amphibia. These animals do not need them, since lipid-soluble substances can be passively excreted through their gills or skin. Land animals, however, must conserve water and therefore have given up gills and semi-permeable skins. Consequently, terrestrial animals have developed enzymes to convert lipid-soluble foreign compounds to excretable products. These enzymes are present in reptiles, mammals, birds, toads and insects. They are also present in semi-aquatic forms such as certain frogs, salamanders and crustaceans.

Animals in ascending the scale of evolution emerged onto land in a number of ways. It is unlikely that the problem would have been solved in the same way more than once, as direction in evolution is one of probability. In accord with this view is the finding that toads and insects appear to oxidize foreign compounds by mechanisms that are quite different from those of mammals and birds. If this concept is correct,

then a powerful tool for studying the mechanism and the sequence of biochemical evolution is available. For example, toads oxidize drugs by dehydrogenation, in contrast to reptiles which have systems which activate oxygen. But meadow frogs, which can live on dry land for 48 hours, metabolize drugs by microsomal systems requiring TPNH and O_2. This suggests that the microsomal systems originated in amphibia and not in reptiles.

Toad and salamander tadpoles cannot metabolize foreign compounds until they have undergone metamorphosis. Of interest is the finding that guinea pigs and mice are born without the ability to hydroxylate barbiturates, dealkylate alkylamines, reduce nitro compounds or form glucuronides. The appropriate enzymes begin to appear in the first week after birth.

Fish can form neither ethereal sulfates nor glucuronides. However, these reactions are present in amphibia and in higher vertebrates. A considerable puzzle, however, is the finding that though fish microsomes have the glucuronide transferase they lack the enzyme required for formation of "active" glucuronide.

Studies on Ascorbic Acid and a New Pathway of Glucose Metabolism

(a) Studies on biosynthesis of L-ascorbic acid which suggested a new pathway of glucose metabolism have previously been reported as follows: D-glucose ——> D-glucuronic acid ——> L-gulonic acid ——> L-xylulose ——> D-xylulose ——> pentose cycle ——> CO_2. Definitive evidence has now been obtained for the occurrence of these reactions in the animal. This scheme, referred to as the glucuronic acid pathway, is important for several reasons: (1) L-gulonic acid is converted to L-ascorbic acid in rats and other animals which can exist without dietary vitamin, whereas man, monkey, and guinea pig lack this enzymatic step; (2) this pathway explains the origin of L-xylulose, the sugar excreted by patients with essential pentosuria; (3) inositol is metabolized to an appreciable extent via this pathway since it is converted to D-glucuronic acid and L-gulonic acid; (4) this pathway may be involved in the synthesis of D-ribose present in nucleic acids.

Of particular interest is the observation that various drugs of widely different chemical structure and pharmacological effect markedly stimulate the metabolism of glucose via the glucuronic acid pathway. Included are such compounds as barbiturates, aminopyrine, phenylbutazone, meprobamate, and the carcinogenic hydrocarbons, 3, 4-benzpyrene and 3-methylcholanthrene. This effect on the glucuronic acid pathway is not related to glucuronide formation but is due to an enhanced activity of this glucose pathway induced by some unknown mechanism. This effect appears to be mediated through the pituitary.

(b) Ascorbic acid is almost completely oxidized in the body to respiratory CO_2. An important step in this series of reactions is carried out by kidney enzymes. A sugar acid, L-lyxonic acid, not previously found in animals, is a product in this reaction. This observation may be a valuable lead towards elucidation of the pathways along which vitamin C is metabolized in animals.

Development of New Drugs

Studies with Butazolidin

Butazolidin (phenylbutazone) is a potent non-steroidal antiarthritic agent but side effects limit its usefulness. Studies with 65 analogs have been carried out in collaboration with Mt. Sinai and Goldwater Memorial Hospitals, New York, and Geigy Pharmaceutical Co., Base.. To date this program has yielded two new drugs. One, a hydroxyl metabolite of Butazolidin, is now in clinical trial; it has potent antirheumatic activity in acute gout and rheumatoic arthritis, and is reported to have less propensity for producing gastrointestinal ulcers than does Butazolidin. The other, a sulfoxide derivative (Anturan), is the most potent uricosuric agent yet described and is about to be introduced for treatment of chronic gout.

The structural requirements for the uricosuric properties of Butazolidin have been outlined. Modifications resulting in compounds of increased acidity have enhanced uricosuric activity, whereas changes resulting in compounds with decreased acidity have little or no activity. It may be inferred that the ionic form of these agents blocks the tubular reabsorption of uric acid. The relationship between uricosuric activity and pKa in the phenylbutazone series is of considerable aid in development of effective uricosuric drugs.

Butazolidin analogs with various substitutions in the benzene rings retain the potent antirheumatic activity of the parent drug whereas substitutions in the butyl sidechain markedly diminish this activity. It had been thought that this loss of antirheumatic activity was associated with a low pKa. But Butazolidin, substituted with an NO_2 group in the p-position of a benzene ring, is highly acid; it is not only strongly uricosuric but has marked antirheumatic activity. It now seems possible that an alkyl sidechain is necessary for high antirheumatic activity. Compounds with various alkyl sidechains are now being tested.

Studies with Flexin

Flexin, a muscular relaxant drug, is an extremely potent uricosuric agent. This is surprising since Flexin is a base while other uricosuric agents are relatively strong acids. As a result of this finding, Flexin is now being used clinically in the treatment of gout. In the course of studies with Flexin, it was found to be converted in vivo to a hydroxyl derivative which possesses potent muscular relaxant properties but no uricosuric activity. The metabolite is more predictably absorbed and causes fewer side effects. Called Paraflex, it has been introduced recently for treatment of disorders associated with peripheral muscular spasm.

Reserpine Substitutes

Reserpine, in small intravenous doses, elicits hypotension, without sedation. On oral administration, most of the drug is hydrolyzed in the gut, and slight variability in the rate of hydrolysis can make a big difference in the total amount absorbed. This may explain the difficulty in controlling blood pressure without eliciting sedation. In collaboration with Ciba, we are attempting to develop analogs that are stable in the gut.

Laboratory of Clinical Biochemistry

The efforts of this laboratory have, as in the past, been centered on various aspects of amino acid metabolism, in its broadest terms. A most interesting finding has been the widespread nature of amino acid decarboxylations. Thus it is now established that in mammalian tissues the amino acids tyrosine and tryptophan are decarboxylated to tyramine and tryptamine. It also appears likely that phenylalanine is converted to phenylethylamine. These pathways which are normally obscured by subsequent metabolism are made evident in animals and patients following administration of the newly discovered potent inhibitors of the enzyme monoamine oxidase (MAO). Other amino acid decarboxylations may be made apparent in this way too. The significance of these amines, both normally and in the pharmacologic manifestations of MAO inhibition, remains to be determined.

It is apparent that routes of metabolism other than by MAO are also important. In the case of serotonin, conversion to serotonin-O-glucuronide becomes the major route and functions very efficiently when MAO is blocked in animals. In man, although a major alternate route apparently exists it does not involve glucuronide formation. However, the presence of such alternate pathways makes it possible to administer large amounts of serotonin orally to animals and patients following MAO inhibitors. By measuring the percentage conversion to the MAO end-product, 5-hydroxyindoleacetic acid, it is possible to determine the extent of MAO inhibition in vivo. Such a test has been applied to patients on MAO inhibitor therapy in an attempt to relate enzyme inhibition to pharmacological effects, particularly postural hypotension. Preliminary studies show a definite correlation.

Tryptamine has been found normally in urine and in animal tissues following the administration of MAO inhibitors. It is not converted to serotonin but is converted to a hydroxylated indoleamine like serotonin. The structure and significance of this hydroxyindoleamine are under study.

Studies on catecholamines also indicate the possibility of an alternate route of metabolism of 3, 4-dihydroxyphenylethylamine (dopamine) leading to 3, 4, 6-trihydroxyphenylethylamine. This product is formed by autoxidation of dopamine and is apparently excreted in animal urine after dopamine administration. Its significance remains to be determined. Conversion of dopamine to noradrenaline has been demonstrated in brain tissue where the tissue catalyst is apparently localized in brain stem areas, as is noradrenaline itself.

There is, at present, disagreement as to which pathway of norepinephrine metabolism is of major importance in vivo. The two demonstrated pathways, MAO and O-methylation, are under study. Methods for assay of compounds formed by the latter route are in process of development. Techniques for inhibiting the in vivo metabolism of norepinephrine by administration of compounds which block either MAO or O-methylation, or both, are under study. It appears that only when both pathways are blocked can the rate of metabolism of norepinephrine be significantly decreased.

Two amino acids which are found only in collagen, hydroxyproline and hydroxylysine, have been under study for some time. The information from these investigations will be valuable in studies on connective tissue formation and pathology. Apparently hydroxyproline can be incorporated directly into collagen in rapidly growing tissues such as chick embryos. Conversion of proline to hydroxyproline has also been demonstrated in cell free systems. Ketoproline, the dehydrogenated analogue of hydroxyproline, has been shown to increase tissue levels of hydroxyproline in animals in vivo. It can do this because it blocks metabolism of hydroxyproline and because it is directly converted to hydroxyproline by some enzyme system, as yet unknown. This compound will be further investigated in vivo to see if it can influence collagen metabolism.

The product of the action of glutamic acid decarboxylase on its substrate is γ-aminobutyric acid (GABA). This amino acid is found in highest concentration in brain and its metabolism in mammalian brain is therefore of great interest. One route of metabolism involves transamidination to yield γ-guanidinobutyric acid. This compound is also found and formed in brain. Like GABA it produces inhibition of central cortical synaptic activity. The significance of γ-guanidinobutyric acid and its further metabolism are under study.

Other routes of GABA metabolism are also of current interest. Incorporation into analogues of pantothenic acid containing GABA instead of β-alanine have been under study. Hydroxylation of GABA to β-hydroxy GABA has been reported, attempts to corroborate this and study its mechanism are in progress.

General Medicine and Experimental Therapeutics Branch -- Section on Clinical Endocrinology

Work on aldosterone - physiologic control of its secretion, relationship to certain disease states, and its metabolism - has been a major effort of this section.

The modification of aldosterone secretion by changes in body potassium was further investigated in balance studies in normal subjects. It was found that the effects of potassium did not operate by producing reciprocal changes in intravascular volume. The effect of expansion of intravascular volume was further explored by infusion of albumin especially prepared by the American Red Cross to contain potassium instead of sodium. In all cases aldosterone secretion appeared to change in a direction reciprocal to that of intravascular volume. Neural pathways in the control of aldosterone were explored in dogs by producing constriction of the inferior vena cava before and after production of lesions. The vagus was thus identified as important for the decrease, but not the increase of aldosterone secretion.

Patients with postural hypotension were found consistently to have lower aldosterone secretion than normal individuals subjected to the same type of stimulus. Patients with renal tubular acidosis were studied and found to lack normal ability to retain sodium and to secrete inappropriately high amounts of aldosterone. A new method for determination of aldosterone was developed and the conversion of progesterone to aldosterone demonstrated in isolated perfused glands.

With the use of the ultrafiltration technique, the plasma protein binding of a large number of steroids was investigated. A new method was developed which revealed that the binding of hydrocortisone is of the order of 90% rather than 99%, the previous estimate from reported studies carried out at unphysiologic temperatures. Studies were begun on the role of hormones in increasing the amount of steroid-binding plasma protein, and of the possible role of this protein in altering metabolism of circulating steroids.

A series of synthetic steroids was investigated for anabolic properties and studies were begun on the effects of halogenated steroids on electrolyte metabolism.

Studies on phosphorus and calcium metabolism were carried out in 11 patients with hyperparathyroidism before and after surgery, and control studies in 6 normal subjects. An estimate was made of the relative value of phosphate clearance, response to phosphate withdrawal, and calcium infusion as diagnostic tests. The defect of calcium metabolism in sarcoidosis was further investigated: the studies show that patients with hypercalciuria and sarcoidosis do not uniformly show sensitivity to vitamin D, increased absorption of calcium, or response to steroid therapy. Attempts to produce radioactive vitamin D were initiated to aid in this phase of the study.

The effect of hypercalcemia and hypercalciuria on the renal concentrating mechanism and on renal sodium conservation were investigated in a number of patients before and after modification of the hypercalciuria by therapy. The inability of these patients to reabsorb water was confirmed and found not to be related to an inability to reabsorb sodium. The presence of the defect correlated better with the urine calcium than with the serum calcium or the therapeutic agent.

The antidiuresis of cirrhosis was investigated and was found not to be related to antidiuretic hormone, but probably to depend on proximal sodium reabsorption: it could be decreased by infusion of mannitol, and reproduced with rigorous sodium deprivation in normal subjects.

Patients with potassium-losing renal disease were studied with metabolic and renal function techniques. Whereas a number of these subjects appeared to have renal tubular acidosis as the underlying disease, others showed the disorder despite normal ability to secrete hydrogen ions. The role of aldosterone in these syndromes is being studied with the use of all agents known to alter aldosterone secretion in the normal state.

In studies in rabbits and dogs the transfer rate of albumin and other large substitutes across arterial walls was measured by the introduction of radioactive labels. The role of absolute pressure, pulse pressure, and alteration of the endocrine status of the animals was tested in acute and chronic studies. Both radioalbumin and radiocholesterol moved out of the proximal aorta much more rapidly than the distal aorta.

The effects of bovine growth hormone in man were studied in a number of obese and normal subjects to determine whether the fat content of the diet would alter response to the hormone. It appears that this

effect, which has been reported for the rat, cannot be reproduced in man. A new method for determination of magnesium and one for the determination of weak beta radiation in aqueous solutions were developed.

--Section on Experimental Therapeutics

The principal projects may be grouped as (1) studies on vasoactive substances, (2) metabolism of amino acids in man, and (3) action and metabolism of drugs.

(1) Vasoactive Substances: Measurement of catecholamines in blood obtained from various sites within the vena cavae was found to be useful in localizing pheochromocytomas in four patients. Increased amounts of m-O-methyl metabolites of norepinephrine have been demonstrated in the urine of these patients. The methoxy analogue of norepinephrine, normetanephrine, was found in each of 4 tumors. Methods for measuring these compounds would be useful clinically and are being developed. Since O-methylation is an important process in the physiologic inactivation of norepinephrine, we are attempting to study this process in man by administering less active catechol compounds and studying the percentage conversion to the methoxy excretory product. The status of this mechanism in primary hypertension and its alteration with drugs is of interest.

Large amounts of serotonin, norepinephrine, 3, 4-dihydroxyphenylethylamine (dopamine) and tyramine (recently) have been found in the banana. Serotonin and tryptamine have been found in several plants. Ingestion of bananas causes an increased urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA) equivalent to the serotonin eaten, no alteration in excretion of free catecholamines, and a considerable increase in acid-hydrolyzable conjugates of catecholamines. Chemical diagnostic studies for malignant carcinoid and pheochromocytoma may give false positive results unless this newly recognized dietary factor is considered.

Monoamine oxidase (MAO) is involved in the metabolism of several biogenic amines. Taking a cue from our findings in bananas, a test was developed for measuring drug-induced MAO inhibition in man, based on the conversion of orally administered serotonin (20 mg) to 5-HIAA. One of the first drugs studied, 1-phenyl-2-hydrazinopropane (JB-516), was found to be a potent MAO inhibitor and concurrently found to be an effective antihypertensive agent (see below). Curiously this agent is pressor in the dog. While the oral serotonin test affords a reliable index of MAO

inhibition in the gut and possibly liver, a method for measuring overall MAO inhibition became desirable.

A sensitive and specific method for measuring urinary tryptamine was devised. Marked increases in the excretion of this amine were observed in patients treated with JB-516. The level of urinary tryptamine appears to be an excellent index of the MAO inhibitory action of investigational drugs. Other amines previously undetected in mammals may be found in the urine of patients when MAO has been inhibited. We have already positively or tentatively identified tyramine, o-tyramine and phenylethylamine under these circumstances.

Since it appeared that amines other than norepinephrine and serotonin might be involved in responses to MAO inhibitors, the pattern of cardiovascular response to various amines is being studied in the dog and in man before and after MAO blockade. Potentiation of the response to dopamine and tryptamine but not to norepinephrine and serotonin were shown in the dog. A marked potentiation of dopamine pressor actions has been confirmed in man. Thus, it is conceivable that dopamine in the sympathetic nervous system may have a role other than simply serving as the precursor of norepinephrine.

(2) Metabolism of Amino Acids in Man: The urinary excretion of bound hydroxyproline (OPR) was found to be elevated in 8 of 10 patients with Marfan's syndrome, possibly reflecting a basic defect in the metabolism of collagen in this disorder. The daily excretion of bound OPR is independent of dietary intake of the amino acid unless it is taken in the form of gelatin. Dietary alterations in OPR have not produced any alterations in patients with Marfan's which are not also shown in normals. The influence of other amino acids and proteins will be studied further and an attempt made to study the body pool of OPR with labelled proline and OPR.

Tryptophan loading in man has been shown to increase tryptamine excretion 2-3 fold. When this was done in patients on JB-516, higher levels of excretion were obtained and the patients complained of a "drunk feeling." Although ingestion of tryptophan is usually innocuous, under MAO inhibition it appears to have definite pharmacologic effects. Previous studies on indoleacetic acid (IAA) have been extended with the finding of increased excretion in several gastrointestinal disturbances other than sprue, including pancreatic insufficiency and blind intestinal loop. Increased IAA excretion in these disorders is considered to be due to altered

bacterial flora in the gut. A report of increased IAA excretion after exercise (Kral et al. 1956) was confirmed but found to parallel urine flow.

(3) Action and Metabolism of Drugs: Chemical agents which are being classified as MAO inhibitors are being evaluated in the treatment of primary hypertension. Of several inhibitors studied, the most promising is the hydrazine derivative JB-516. A marked orthostatic lowering of the blood pressure was produced in 9 of 10 hospitalized during short-term therapy with single daily doses of the drug (25-50 mg). A favorable therapeutic response was obtained in a larger group of clinic patients over periods of 2 to 8 months. An interesting and temporary side effect was development of a defect in red-green color discrimination in six patients receiving high doses (25 mg) of the drug. The orthostatic hypotension resembles that seen with ganglionic blockade but parasympatholytic effects are negligible. The association of MAO inhibition with hypotension is becoming increasingly evident but enzymatic and physiologic studies are continuing with a variety of inhibitors. Compounds containing the hydrazine prosthetic group also interfere with the metabolism of pyridoxine and thereby may alter the decarboxylase and transaminase pathways of amino acid metabolism. This is a separate problem for study.

During the past year, we have instituted broader therapeutic evaluations of MAO inhibitory drugs in hypertension at George Washington University Hospital with Drs. I. Tamagna and H. Orvis. The combination of chlorothiazide (1.0 gm/day) and iproniazid (75 mg/day) was found in a precise study to constitute an effective anti-hypertensive regimen in a small group of patients.

An attempt was made to evaluate the effectiveness of iproniazid in angina pectoris in 10 patients, with inconclusive results. A double blind study at D. C. General Hospital in 13 patients was also inconclusive. We are currently studying JB-516 in this condition at the NHI, D. C. General Hospital and Baltimore PHS Hospital.

Preliminary studies suggest that the short-acting cholinergic drug, edrophonium (tensilon) may be useful in treatment of paroxysmal supraventricular tachycardia.

--Section on Cardiodynamics

The activities of this section over the past year have shown an increasing use of biophysics in clinical cardiopulmonary investigation. A great portion of the total energy of the Section has been devoted to the development and validation of instrumentation for the direct measurement of physical processes in the living intact human subject. A method for the instantaneous and continuous measurement of aortic blood velocity was developed for use in man. This advance has opened the way to many extremely important goals, e.g., the measurement of the horsepower of the heart, the kinematics of cardiac ejection, power loss at diseased valves, distributed impedances and junctional admittances in the vascular tree. Measurements of this kind will be necessary to define the abnormal physical properties and energetics of the vascular system in myocardial disease, coronary attenuation, arteriosclerosis, hypertension, and related conditions.

Pilot studies in animals, using the blood velocity catheter, are in progress to determine the effect of interventions on the coronary circulation. Acute attenuation of the coronary circulation either by ligation or embolization of the coronary tree produces dramatic and reproducible changes in the blood velocity curve. Methods are being developed for producing chronic coronary insufficiency in dogs.

Pilot studies in dogs are in progress to determine the pressure-diameter relationships of various parts of the systemic and pulmonary vascular bed so that inferences regarding flow can be made from the blood velocity curve. When these studies are completed, the problem of systemic and pulmonary vascular resistance can be better evaluated. Preliminary data indicate that although there is a marked divergence between the shape of the pressure and velocity curves in the aorta, their shapes become almost identical as either the systemic or pulmonary capillary bed is approached. This indicates that the arteriolar-capillary-venule bed probably behaves as a pure resistance without significant reactance.

Vascular resistance in the pulmonary bed is being studied both by the catheter blood velocity technique and by conventional dye dilution curve methods to establish (1) comparison of the methods, (2) the effect of intrathoracic pressure on resistance, and (3) the effect of various pharmacological agents on resistance. These studies are being carried out both in man and in animals.

Since the direct measurement of the intrathoracic pressure in man is hazardous and in itself alters the normal function of the lung, an active program is in progress to establish the relationship of intrathoracic pressure to intraesophageal pressure. The intrathoracic pressure is necessary for determination of the transmural stress of the intrathoracic structures in most of the foregoing studies.

New methods of studying the mechanical behavior of the lung were developed. Detailed studies of the unified pressure-flow-volume-time relationship of the living human lung were carried out in normal, cardiac and emphysematous subjects. A relationship between the maximum achievable expiratory flow and degree of lung inflation was discovered which theoretically has far-reaching physical and physiological implications of importance.

The ground work is being laid for an even stronger biophysical approach to the problems of clinical cardiopulmonary investigation. New avenues of instrumentation and computer techniques are being explored.

Modest studies in what might be called the quantitative morphology of the heart are continuing. These include: (1) the use of biochemical partitioning methods to define the quantity and distribution of connective tissue in heart muscle. It is not widely appreciated, for example, that myocardial hypertrophy is accompanied by a striking increase in myocardial connective tissue, in some conditions accounting for one-third of the increased heart weight. The biochemical methods for this project are being refined; (2) studies of the biochemical and physiologic properties of hypertrophied muscle, using experimentally produced skeletal muscle hypertrophy as the prototype. This has proven to be a much more difficult project than had been anticipated because each contraction of the muscle must be maximally loaded; (3) analysis of the architectural deformity of the heart in congenital heart disease using special methods for dissecting the myocardial muscle layers and topologic methods for formulating the kinetics of the developmental abnormality.

Electrocardiographic research has had three directions: (1) a study of the changes in QRS potentials on closure of atrial septal defects. It had not previously been well recognized that nearly instantly with obliteration of the interatrial shunt there is a reduction in the duration of QRS potentials, as if the right ventricular conduction defect were reversible with reduction of right ventricular diastolic volume. The implications of

this are being further pursued. (2) A study of the electrical deformity of the Wolff-Parkinson-White syndrome has been completed and published. The interesting finding was the high incidence of abnormal ventricular conduction in this syndrome, the characteristics of which shed light on the probable mechanism of the syndrome. (3) Considerable effort has been expended to increase our supply of autopsy-controlled ECG data for various age groups and disease entities. We have well beyond 1,000 cases now in this file. The analysis has concentrated, so far, on defining normal ranges of ECG data in infants and children, and in the age group beyond 60 years. Collaboration with Dr. George Manning of the RCAF will augment our data for the third and fourth decades. This will be the first statistically satisfactory analysis that has ever been made of autopsy-controlled and age-controlled ECG data.

Surgery Branch

The clinical investigative projects originating in the Surgery Branch during the past year have again utilized, in general, patients with various forms of congenital heart disease and those with acquired valvular disease. A limited number of normal controls have been admitted.

An important group of studies are those clinical and experimental ones involving the detection and quantification of circulatory shunts. Previous reports have detailed the usefulness of inert gases such as nitrous oxide and krypton^{85} in the detection and localization of left-to-right shunts. These methods are now established and are being widely adopted in clinical laboratories throughout the country. By the application of a mixing equation the results of a nitrous oxide or Kr^{85} test may also be used to determine the magnitude of a left-to-right shunt and the validity of this method was proved when metered flows were measured in animals as simultaneous nitrous oxide tests were carried out. The isotope Kr^{85} has proved to have other interesting applications in the study of shunts. This gas is slightly soluble in saline solution and when injected into the venous circulation in the normal animal or patient it is virtually completely eliminated by a single passage through the lung and only small quantities of it appear in the arterial circulation. When a right-to-left shunt is present, however, large quantities of the gas appear in the arterial blood. This technique provides a new and extremely sensitive method for the detection of veno-arterial shunts. When such solutions are injected into the left heart in the normal individual, the expired air contains the gas immediately in high concentration but for only a brief period. When a left-to-right shunt is present, however, the concentration of the isotope in expired air is lower and its time concentration is greatly prolonged by pulmonary recirculation. Thus, yet another means is afforded for the detection of arteriovenous shunts. All of these techniques are being applied in selected patients and should greatly increase our ability to characterize precisely those forms of congenital heart disease with abnormal intra- or extracardiac vascular communications.

Indicator substances, both radioactive and colored, are being evaluated as new tools for determining the patency of portacaval anastomoses. When the substances are injected into the spleen in the normal dog or in a dog with an occluded anastomosis the substances appear late and in low concentration in systemic arterial blood since they are delayed by passage through the capillary bed of the liver. When the anastomosis

is open, however, the indicator immediately appears in arterial blood and in high concentration. This technique has been fully evaluated in the experimental animal and is being used in patients operated upon for esophageal varices. The indicators may also be used to determine and perhaps quantify valvular regurgitation. In the course of cardiac catheterization the indicator is injected into a chamber as a sample is simultaneously drawn from the next most proximal chamber. When the valve is competent the indicator is found proximally only after normal recirculation has occurred. But when regurgitation is present it is immediately detected. This study is a routine clinical application and attempts are being made to provide it with a quantitative basis.

The Section on Cardiology has carried out a group of studies on the physiologic effects of digitalis in patients. In normal subjects, the effects of rapid digitalization on cardiac output and central blood volume were measured. Preliminary results indicate that the work of the left ventricle and the cardiac output and central blood volume are significantly decreased. Patients with atrial fibrillation have also been studied to determine the dose of digitoxin necessary following the rapid administration of ouabain. Results of these studies are as yet incomplete as various parameters of preliminary dosage are necessary. In similar patients the relationship of the effect of digitalis on the heart rate has been studied before and after vagal blockade with atropine. It is hoped that the interrelationships of digitalis dosage to potassium, calcium, and norepinephrine may be determined. It seems, from the preliminary work, that potassium increases the dose of digitalis necessary to achieve a given ventricular rate.

An important project of the Section on Cardiology is a long-term study of ventricular function in essentially normal patients. Cardiac output and systemic pressure have been measured before and after the infusion of large quantities of blood with and without ganglionic blockade. The data indicate that Starling's law of the heart applies when the regulatory mechanisms of the body are blocked. Another experimental physiologic study is concerned with whether the heart muscle has "tone" and to determine whether the pressure-volume curve of the ventricle can be modified by the administration of various drugs or by changing various hemodynamic factors. The basic tool in this study has been a method for determining the end diastolic fiber length with a Cushny lever and the end diastolic volume by infusion studies following sacrifice. A characteristic pressure volume curve has been established for the dog ventricle. Distensibility was not changed by the administration of epinephrine or

norepinephrine. Other studies have been concerned with extending previous physiologic studies in experimental mitral insufficiency. An experimental lesion was produced in which regurgitant flow could be metered and changed at will. With various regurgitant flows, measurements of the left atrial pulse contour were made and the effects of vasopressor agents were studied. Preliminary observations are that vasoxyl has a much more pronounced effect in elevating the left atrial v point pressure than does norepinephrine when mitral insufficiency is present. It is possible that the clinical use of these drugs in emphasizing the hemodynamic effect of mitral insufficiency will be changed because of the results of these experiments.

A large percentage of the clinical and laboratory work of the unit has been devoted to the experimental study and clinical application of extracorporeal circulation. Within the past year a new pump oxygenator has been designed and constructed, evaluated in the laboratory and applied in the treatment of 75 patients undergoing open cardiotomy. Further refinements of the apparatus will permit precise control of its internal volume and the temperature of the blood. In the course of the clinical work with this apparatus interest has been renewed in the effects of potassium citrate and other cardioplegic agents on the function of the heart. While the heart is stopped with potassium, perfusion studies show there is no measurable oxygen, glucose, lactate or fatty acid utilization. With "arrest" with acetylcholine these substances are utilized but at a greatly reduced rate. The function of the heart has also been studied by the construction of ventricular function curves in animals before and after cardiac arrest with potassium and acetylcholine. Both of these drugs have been found to depress ventricular function severely when periods of arrest exceed 10 minutes and a comparison of these agents with anoxic arrest is presently being made to determine which technique seems most suitable for clinical use.

Pathologic pulmonary changes were noted in a number of patients and animals following extracorporeal circulation. The etiology of these congestive changes was shown to be elevated pulmonary venous pressure which resulted from bronchial venous return into the intact and arrested heart. The demonstration that the elevated pressure and lung damage could be prevented by venting the heart throughout perfusion and cardiac arrest has been proved to be of value in clinical practice. Another clinical study of great help in the management of patients has been the application of the plasma thrombin time to determine whether circulating

anticoagulant remains after the administration of protamine. In 25 patients it was shown that this study provided the most useful and reliable estimate as to the adequacy of protamine administration.

A number of physiologic studies concerned with whole body perfusion are in progress. Hepatic blood flow is being measured in dogs during bypass at various rates of perfusion. Hepatic blood flow does not change if perfusion at normal cardiac output is maintained but if perfusion rate decreases the liver flow is an increasing percentage of total flow. These experimental studies are being correlated with the measurement of liver function in patients before and after perfusion. In the laboratory the heart lung machine provides a useful tool for the study of hemodynamic factors when the heart is completely excluded from circulation. For example, during bypass with the heart stopped, digitalis has been given and it has been shown to have a marked effect on peripheral resistance which is not blocked by hexamethonium. Similarly, changes in peripheral and organ blood flow may be measured in a similar fashion. With the onset of complete bypass there is an immediate fall in peripheral resistance which then gradually rises during the remainder of the bypass. The effects of hypoxia, various drugs and carbon dioxide on peripheral flow and resistance during perfusion are under study.

Numerous previous reports have dealt with the technique and application of left heart catheterization in the assessment of patients with various forms of heart disease. As of this date, 900 transbronchial left heart catheterizations have been performed without a death or serious sequel. There has been increasing application of left heart catheterization in the study of patients with congenital heart disease. Patients with congenital aortic stenosis have been of particular interest and their assessment by this method has proved a valuable adjunct in management. Indicator-dilution curves with left heart injection are also being carried out with increasing frequency. Other methods of left heart catheterization are also being used. Percutaneous puncture of the left ventricle has been carried out in nearly 100 patients. This technique is particularly applicable in young children and in those patients with severe mitral valve disease in whom the catheter cannot be passed into the left ventricle bronchoscopically. In many instances a combined transbronchial and percutaneous approach has been used. Selective angiocardiography by means of direct puncture of the left atrium or left ventricle have been carried out in a limited number of patients. This technique shows great promise in the evaluation of mitral insufficiency, the localization of aortic stenosis, and the detection of left-to-right shunts.

Gerontology Branch

During the past year, investigators concerned with the basic biology of aging have been recruited and placed on duty. A total of ten new laboratories has been equipped and will be ready for occupancy by January 1, 1959. New research programs in cellular physiology, experimental embryology, the mechanisms of intermediary metabolism, biophysics and molecular biology have been initiated. These studies will increase our knowledge about the basic biology of aging. For example, aging in a simple organism (Campanularia) has been found to be associated with a progressive loss of an enzyme important in energy transformations (ATP). It has also been found that with increasing age there is an accumulation of highly insoluble granules in heart muscle in the human.

Physiological and psychological tests have been administered to a group of volunteer subjects still living in the community. Observations on these "elite" subjects will permit us to evaluate the effects of socio-economic and educational status on aging phenomena in the human.

The latency of certain reflexes is significantly increased with age. This lowered excitability is believed to be due to a reduction in excitatory processes in the central nervous system and may have important implications for other psychological processes. Short span perceptual memories are more susceptible to interference in old subjects than young.

More detailed findings are listed below:

Basic Biology

1. During growth, there was a marked reduction in the rate of oxidative phosphorylation by liver tissue in rats. After reaching adult levels, there was no significant change with increasing age. Rates of oxidation and the amount of phosphorus esterified decreased with decreasing amounts of mitochondrial nitrogen in the test system.

2. Age differences in the capacity of the liver of the rat to re-synthesize specific proteins was tested by following the rates of regeneration of proteins following periods of protein-free feeding. Following protein depletion, the coucentration of d-amino oxidase and cholinesterase fell markedly in the liver of both old and young rats. Following

repletion, the levels of these enzymes returned to control levels in the old animals. Old rats were as capable as young animals in restoring plasma protein levels after protein depletion. Thus, there is no evidence that age interferes with the ability of the liver to synthesize these specific proteins. The effect of protein depletion on the rate of synthesis of other enzymes is being studied.

3. Protein depletion did not induce the same type of changes in liver enzymes of the rat as did aging.

4. Using transplantation, extirpation and grafting techniques, it has been found that the amount of "juvenile hormone" present in the developing cockroach is dependent on age. The effect of age of a tissue on its sensitivity to hormonal influences is also under investigation. By repeated transplantation into successive adult hosts, certain tissues (organ discs) of the fruit-fly can be maintained for long periods of time without undergoing final differentiation, since the adult fly does not contain the hormone necessary for differentiation. By transplanting "aged" discs back into a larval host, the effects of age on subsequent development will be studied.

5. Equations have been derived for zero order and first order chemical reactions in tissues which will predict the distribution of metabolites in the tissue surrounding a capillary.

6. The light transmission changes induced in metabolizing systems of luminous bacteria has been shown to be due to a change in scatter rather than absorption. An adaptation of a light integrator has been developed for the Carey Model 14 Spectrophotometer which permits the measurement of the absorption spectra of light scattering biological materials with simplicity, proficiency and accuracy.

7. The general theory of mortality rates has been extended to include estimates of the maximum rate of inherent aging, the minimum rate of environmentally induced aging, and the amount of reserve functional capacity at any age. Preliminary calculations, based on mortality rates in "good" and "poor" environments, predict a maximum life span in the human of approximately 140 years.

8. Short exposure to high temperature (36° C.) does not appreciably alter mortality rates in Drosophila, but there is a permanent effect induced by exposure to temperature above 38° C.

9. There is a linear increase in the percentage volume occupied by lipofuscin pigment ("age pigment") from 0% at age 10 years to about 4% at age 90. The percentage of the myocardium occupied by muscle fibers is about 60-70% in the 0-10 year age group and drops to 40% by age 40 without much change thereafter. The amount of pigment in the heart is not significantly correlated with the pathological diagnosis of the autopsy material used in these studies.

10. Older hydranths of the colonial hydroid, Campanularia, are comparable to young ones with respect to their ability to catch food and to transmit it to other members of the colony. However, the concentration of ATP of hydranths diminishes during aging and reaches vanishing concentration at the time of regression.

11. Studies of the structure of hemoproteins indicate that the heme group in hematin is not asymmetric but becomes a center of asymmetry in cytochrome-c, hemoglobin and myoglobin. When hemoglobin is cleaved into half-molecules, rotatory dispersion studies indicate that the heme asymmetry is lost. This shows that the four heme groups in the hemoglobin molecule are so placed that both sides of the heme group are identical in the half-molecule.

12. The mechanisms of action of metal catalyzed enzymatic reactions have been studied. The enolase and aconitase reactions have been selected as examples of enzymatic reactions that accomplish very similar molecular transformations but require different metal ions, i. e., magnesium and iron (II) respectively. The substrates of both reactions have been found to produce metal complexes of varying stabilities, but evidence for non-enzymatic reaction has been obtained only for the interaction of iron with the aconitase substrates.

Human Physiology

1. Studies on age changes in body form and composition have shown that the age decrement in thoracic height was attributable to an increase in spinal curvature rather than to compression of intervertebral discs.

2. Improvements have been made in the instrumentation and technique of measuring body volume by helium displacement which permit reproducibility of 0.3% for either humans or standard volumes.

3. The average latency of the plantar flexor and superficial abdominal reflexes was greater in old than in young subjects. In elderly persons, the lowered excitability of these reflexes, as determined by increased latency, is believed to be due to diminished central excitatory processes which is reflected in effector systems governing reflex activity, rather than those related primarily to simple voluntary motor performance.

4. Studies of the mechanics of limb movement have emphasized the relation between amplitude of swing and mechanical efficiency for a series of rapid back and forth movements of the arm around the shoulder as an axis. The greater mechanical efficiency at high displacements is interpreted as the result of a greater proportion of "free swing" at these displacements. The greater efficiency of young as compared with old subjects is interpreted as the more simultaneous recruitment of greater numbers of muscle fibers in the muscles of young subjects. This is associated with the observed greater accelerations and higher swinging rates in the young than in the old subjects.

5. Methods for the estimation of dextrans of different molecular weights have been standardized and a number of dextran fractions with a restricted band of molecular weights have been prepared by reprecipitation techniques for calibration purposes.

6. The time course of the development of increased urine concentration following water deprivation has been followed. Osmolar U/P ratios ranged from 1.59 to 4.28 with an average of 3.08. In almost half of the subjects studied, water deprivation had no systematic effect on urine osmolality. The factors involved in this failure to respond are being investigated.

7. The maximum osmolality of the urine diminishes significantly with age in the rat. In the rat, diminished osmolality of the urine is associated with an increased proteinuria.

8. In order to test the effects of socio-economic and educational status of subjects previously studied from the Infirmary population of the Baltimore City Hospitals, a sample of individuals (aged 30-96) still living in the community has been recruited. These subjects spend 2-1/2 days in the Baltimore City Hospitals and are subjected to an extensive battery of physiological and psychological tests. All are recruited on

the basis of return visits at intervals of 1-2 years. Facilities of the laboratory limit the program, at present, to testing two subjects per week.

Psychological Studies

1. Short span perceptual memories are more susceptible to interference in old subjects than in young.

2. Apparatus has been constructed to investigate the effect of stimulus complexity on the age decrement in reaction time.

3. The relationship between alpha frequency of the EEG and reaction time is being studied.

Program Development

During the coming year, studies on basic biology will include: separation of particulates from old and young cells, with the aim of determining their functional capacities; a further characterization of similarities and differences between protein depleted and aged animals; characteristics of regenerating and hypertrophying tissues in young and old animals; the basic mechanisms of oxidative phosphorylation; the influence of structure on function in biologically important compounds; characteristics of aging cells grown in tissue culture; factors involved in cellular differentiation and those concerned with growth limitation; the chemical nature of "age pigments," and the basis for the loss of tissues and cells in aging animals.

The program in human physiology and psychology will be concerned with age differences in the permeability of the renal glomerulus; maximum concentrating capacity of the kidney; body composition; mechanics and efficiency of body movement; functional responses of the cardiovascular system to standardized stresses; reflex excitability; learning ability; reaction time; susceptibility to distractions in psychological performance; attitudes toward aging; central motive states, and their influence on performance.

Additional functional tests will be performed on the volunteer subjects recruited from the community.

PHS - NIH
NATIONAL HEART INSTITUTE
Calendar Year 1958

Serial No. NHI - 1

Administration
(Organization)

The National Heart Institute, under the National Heart Act, is charged with the responsibility of: the conduct of research relating to the cause, prevention, and methods of diagnosis and treatment of diseases of the heart and circulation; assisting and promoting such research by other public and private agencies; coordinating all research results and promoting their application; providing training as necessary; and assisting local and State agencies.

The accomplishments of the Heart Institute administrative offices, from the standpoint of both program direction and administrative services provided, are most properly reflected in the achievements of the component units of the Institute: the Grants and Training Branch; the Intramural Research Branch; the Technical Services Branch, including the Heart Information Center, Epidemiology, and Biometrics Research; and the Heart Disease Control Program. Contributions to the research program by these units are described elsewhere in this report.

The administrative function is constantly being re-evaluated in terms of how well it assists the operating people to do their jobs well. Following is a list of the changes and developments in administrative areas during the year 1958 which, it is hoped, have improved the overall functioning of the Heart Institute:

1. As indicated in our 1957 report, the location of the Heart Information Center in Bethesda, Maryland in the Progress Building proved to be a tremendous handicap to the staff of the Institute. This activity has been returned to the grounds of the Institute and is now located in Building 3. It has been proven that the location of the Heart Information Center off of the grounds presents numerous difficulties in the gathering and assimilation of scientific information.

2. The Center for Aging Research, previously occupying space in Building 3 and formerly administratively under the Heart Institute, has been transferred to the new Division of General Medical Sciences and is no longer a part of this Institute.

3. In order to improve communications between the Information Center and other important segments of the Institute, Information Specialists have been physically located in the Research Branch and in the Grants and Training Branch.

4. Dr. William J. Zukel, formerly Assistant Director of the Institute has been transferred to the Office of the Surgeon General. Dr. Luther L. Terry, formerly Chief of the Laboratory of General Medicine and Experimental Therapeutics has been appointed to succeed Dr. Zukel as Assistant Director of the National Heart Institute.

5. In keeping with the Department's long range objectives to foster and encourage scientific talent, the Heart Institute established a scientific program through which high school students with outstanding records in science were permitted to work in our laboratories during the Summer 1958. The students performed regular laboratory experiments commensurate with their level of maturity and served without pay. This program earned the unanimous approval of both the students and the preceptors, the latter being members of the professional laboratory staff. It is anticipated that a similar program will be put into effect in the Summer of 1959. In addition to this program, our regular Summer employment program for undergraduate students pursuing courses in science and for medical students was in effect. From the standpoint of the Institute, the total Summer employment program provided an intelligent, partially trained, work force which stimulated a number of students, many of whom will undoubtedly seek permanent employment at N.I.H. upon graduation.

6. Two new activities have been set up under the Office of the Director. The first is the establishment of the Memphis Epidemiology Section which will conduct studies involving the incidence of various types of cardiovascular disease in the white and colored races. This is a study in cooperation with the facilities of the staff of the University of Tennessee School of Medicine.

The second activity is the establishment of a Geographic Pathology Section under Dr. James Hundley. Dr. Hundley is assuming the responsibility of investigating the possible effect of geographic location on the incidence of cardiovascular disease. Since both of these activities are newly initiated, individual reports are not being submitted.

Under Study

1. A management survey is being conducted to determine the feasibility of establishing an Office Services Section in the Office of the Director under the supervision of one individual to carry out the responsibilities of travel, supply, central files and general housekeeping. It is anticipated that this proposal will result in better internal management.

Serial No. NHI - 2

PHS - NIH
NATIONAL HEART INSTITUTE
Individual Project Report
Calendar Year 1958

BIOMETRICS RESEARCH SECTION

During the calendar year of 1958 the Biometrics Research Section devoted all its attention to providing statistical services for the Heart Disease Epidemiology Study at Framingham, Massachusetts. The fourth round of biennial examinations of the Framingham study population was completed in July, and the results were immediately made available in tables and slides to show some factors associated with the development of coronary heart disease in the six-year follow-up experience in the Framingham Study. This material was presented by Dr. Thomas R. Dawber at the World Cardiology Congress at Brussels, Belgium, in September, and by Dr. William B. Kannel at the annual meeting of the American Public Health Association in St. Louis, Missouri, in October. A paper on blood pressures in the Framingham study group was prepared for presentation by Dr. Abraham Kagan to the Conference on Hypertension which met in November. Preliminary analysis of diet information from the Framingham Study was prepared for a meeting of the American Institute of Nutrition early in 1959.

The fourth exam round was very successful and the completion of the work at Framingham reflects very highly on the entire staff there. Expressed as a percentage of the group available for Exam IV, follow-up for Exam IV was 96.3 percent complete. Honored for superior performance were three secretaries at the Framingham Heart Clinic, Lorna P. Lyell, Dorothea M. McElholm, and Patricia McNamara, who divided a $375 superior performance award.

Mr. Tavia Gordon, who was acting as Chief of the Biometrics Research Section, resigned at the end of October to join the staff of the National Cancer Institute. However, in the last two months of the year Mr. Gordon drafted a paper, "Some Methodological Problems in Long-Term Studies of Cardiovascular Disease," and presented it at the meeting of the American Statistical Association in Chicago, December 29. Co-authors of the paper were Mr. Felix E. Moore, formerly Chief of the Biometrics Research Section and now Professor of Biostatistics in the School of Public Health, University of Michigan, and Dewey Shurtleff, who joined the staff of the Section as statistician in November, 1957. The paper reviewed the ten years' experience in the Framingham Heart Disease Epidemiology Study in terms of biases in initial response and biases in follow-up by clinical and other means. The probable effects of these on analytical results and the implications for longitudinal studies in general were reviewed.

NHI - 1.1
NATIONAL HEART INSTITUTE
Individual Project Report
Calendar Year 1958

Heart Information Center

Under the terms established in the National Heart Act of 1948, the Heart Information Center is charged with the responsibility of planning and executing a diversified program of information and education on diseases of the heart and circulation. The Center is designed to serve two consumers. One, the general public. The other, the professional working in the cardiovascular field.

The following pages reflect something of the scope and nature of the work of the Heart Information Center during calendar year 1958.

Exhibits

NHI-AHA Cooperative Program Exhibit: A 30-foot joint exhibit was created to highlight 10 years of cooperation between these two organizations in research, education, and community services. The exhibit was introduced in September to some 3,000 physicians and scientists gathered in Brussels, Belgium for the Third World Congress of Cardiology. In October the exhibit was again shown to 3,000 physicians and scientists during the Scientific Assembly of the American Heart Association in San Francisco. For the next few years this exhibit will be used intensively for professional groups throughout the country.

NHI Exhibit Program: Two new exhibits for professional audiences were completed by HIC for the Heart Disease Control Program. These were widely shown to medical groups throughout the country. One exhibit depicts the relation between sore throat and rheumatic heart disease. The other presents a concise statement of the latest treatment schedules for prophylaxis.

Last year's campaign to disseminate information about strokes has been developed further by an exhibit created this year entitled "The Stroke Patient is a Challenge to the Whole Team." It has been presented at several meetings during the year. In addition, Heart Information has participated in two large high-school science fairs by presenting the exhibit "The National Heart Institute: A Medium for Service." Members of the Center staffed a PHS exhibit at the New York Health Show and the exhibit on the National Institutes of Health during the Washington meeting of the American Association for the Advancement of Science. Consultation on exhibits was given freely during the past year to heart associations and others.

Reports

Preparation of regular and special reports in whole or in part was an important continuing function of the Center. These included the

regular weekly report concerning items of interest, an important research accomplishments and program developments for information of the Director, NIH, the Surgeon General, PHS, and others; the annual report of the Heart Institute for publication in the full NIH annual report, weekly reports itemizing selected informational activities for the Office of Research Information, NIH, and the PHS Information Office; and an annual report on the activities of Heart Information Center. Among special reports prepared were narrative summaries for the Secretary of DHEW on heart surgery, rheumatic fever, arteriosclerosis, and hypertension; reports requested by members of Congress on the Minnesota and Alabama contributions in heart research; reports on outstanding research accomplishments of selected NHI scientists; a report on "International Medical Research," highlighting foreign contributions to present day knowledge in the field of cardiovascular medicine, for the Senate Committee on Government Operations, and finally, a report describing and evaluating a year's traineeship conducted by HIC for the Office of Research Information.

In addition to these reports, the Heart Information Center participated extensively in development of materials required for budgetary and appropriation matters. Included among these documents were the Director's opening statement for hearings on the Institute's fiscal 1960 appropriation; a compilation of highlights of research progress made in Institute-conducted and grant-supported investigations during the 1958 calendar year; a compilation of the highlights in program development which occurred during the year; a compilation of heart research highlights covering the first quarter of 1958, required for the Senate hearings on the fiscal 1959 appropriation and a special status report on hypertension submitted during these hearings.

Publications

Several new publications were issued during the year. Highlights of Heart Progress - 1957, a compilation of items on selected research findings reported during the year from investigations conducted and supported by the Heart Institute, was particularly useful as a means of supplying information on recent developments in cardiovascular research. Written in sufficiently non-technical language for understanding by the general public, it was also of interest to professionals. Also released was a 110-page bibliography, Research Publications of the National Heart Institute, which listed the technical publications of Institute scientists published in professional journals during the period January 1953 through June 1958. Other publications written and published during the year were A Brief Summary of Programs of the National Heart Institute, Working Together...A Message from the NHI and AHA, Some Recent Advances Against Heart Disease, The Role of NHI in International Research, and Public Health Service Support of Cardiovascular Research, Training, and Community Programs. Also issued this year as a cooperative venture with the AHA was the new version of Cardiovascular Disease in the U. S.: Facts and Figures.

Speeches and Articles

Speaking texts and narrative outlines were prepared for a number of speeches given in conjunction with various events and occasions.

these were addressed by the Director of NHI at the opening of the Cardiovascular Research Institute at the University of California, at the Albert Lasker Medical Journalism Award Luncheon, and the CDC Conference for Teachers of Veterinary Public Health and Preventive Medicine and Public Health Workers; by the Assistant Director of NHI at the Alabama Conference on Handicapped Children and the New Jersey Cardiac Surgery Club; by the Surgeon General of PHS at the meeting of the National Advisory Heart Council Alumni Association; by members of Congress before the Washington Heart Association and the Heart Surgery Club of Rhode Island, and by Dr. William P. Shepard to the AMA Scientific Assembly. Special materials prepared by the Heart Information Center that were published during the year included an article bylined by the Director of NHI on "Help and Hope for Children's Hearts" which appeared in National Parent-Teacher; the Foreward by the Director of NHI to the volume of proceedings of the Conference on Intracardiac Surgery; a summary of 1957 progress against heart disease for the World Scope Encyclopedia Yearbook; and an article in Minnesota Lancet bylined by the Associate Director of NHI on "Heart Disease - A World Health Problem".

A background study on "Food Fads" was prepared with a view to possible use in connection with diet recommendations. Also on the subject of diet, a short information statement on the use and dangers of salt substitutes was written and disseminated among paramedical professional people concerned with the care of patients on sodium restricted diets. Three background studies were completed on atherosclerosis, stroke, and coronary artery disease. Based on current periodical literature, these were written to serve Heart Disease Control Program physicians as source materials for speeches and local presentations.

Services to Science Writers

Background material on cardiovascular disease subjects was furnished, special information was obtained and provided, and interviews with NHI scientists and staff members were arranged for many science writers, reporters, and others. Such services, in addition to aiding outside writers in developing stories or completing medical reporting assignments, helped assure the accuracy of material published in mass-circulation outlets. Illustrative of the products to which such Heart Information Center services contributed, were articles by Ben Pearse in the Saturday Evening Post, Francis Bello in Fortune, Eugene Fleming in Cosmopolitan, and Howard Simon in Science News Letter. Among other publications serviced during the year were Time, Newsweek, U. S. News and World Report, Scope Weekly, Medical News, Drug Research Reports, and the Wall Street Journal. Cooperation was also given to science writers of various news services and organizations, including the Associated Press, United Press, NEA, Science Service, Scripps-Howard, and Voice of America, and those on the staffs of several large metropolitan papers. In addition to furnishing requested information and material, photographs were supplied and arrangements made for taking photographs for a number of newspapers and magazines. Background information was provided and interviews were arranged for Harry Neal, author of the forthcoming book, Your Career in Medical Research.

Press Releases, Radio, Television

Sixteen press releases were issued during the year. Twelve of these reported research advances made in Heart Institute laboratories, three announced appointments to membership on the National Advisory Heart Council, and one gave background information concerning an Institute scientist who won a major award for research achievement. Five of the releases involved cooperation with scientific organizations, four being released at the annual meeting of the Federation of American Societies for Experimental Biology and one by the American Association for the Advancement of Science. In addition to the releases, two background statements were prepared on Heart Institute research investigations involving normal volunteer subjects, for issuance to the interested press and others through the Clinical Center Information Office. Working through the media of radio and television, Heart Information Center arranged interviews for the Director, NHI, on such programs as NBC's "Nightline" and "Today" and Voice of America's "World Wide English Show"; for NHI's Assistant Director on "Ask-it-Basket"; and for an NHI visiting scientist on the show, "In Our Town". Assistance was also given in coordinating a heart surgery telecast from NIH to an audience at the recent AAAS convention.

Inquiry and Reference Services

Numerous requests for information from the general public, individuals in medical and health professions, and private and public agencies and organizations were received and expeditiously handled during the year. Many of these were effectively answered by furnishing pertinent leaflets or other appropriate publications; others required special compilation of information or material, consultation with scientists, or preparation of reading and reference lists. The reprint reference collection maintained by the Center, containing papers on research findings resulting from NHI conducted and grant-aided investigations, has proved increasingly useful in connection with information activities. The up-to-date card files of references on Institute-connected publications currently appearing in medical and scientific journals have been particularly valuable in facilitating research reporting.

Specialized Information Programs

Several specialized programs were designed to give information to visiting groups. Among these were the Virginia Health Officers Program; a training session for a group of postgraduate cardiovascular nurses; programs for members of the Social Legislation Information Service, the World Health Organization, the American Association for the Advancement of Science, four State heart association groups, and a group of award-winning students. These programs included laboratory demonstrations, lectures, tours, and meetings with scientists who have interests in common with the visitors.

Thirty-four professional key representatives from State heart associations participated in four NHI heart seminars as part of their orientation and training for heart association work. Through three years of these seminars, a reservoir has been built up of heart association people throughout the country who continue to exchange information with and look to the Heart Information Center for various information services

Department of Health, Education, and Welfare
Public Health Service
National Institutes of Health

Serial NO. 4

I. RESEARCH PROJECT DESCRIPTION

DATE: Calendar Year 1958

1. DIVISION
National Heart Institute

2. BRANCH
Technical Services

3. SECTION
Epidemiology (Framingham)

4. DIVISION PROJECT NUMBER

5. PROJECT TITLE

6. INVESTIGATOR
Dr. Thomas R. Dawber, Dr. William B. Kannel, Dr. Nicholas Revotskie, Miss Georgiana Pearson, Dr. Abraham Kagan, Dr. Joseph Stokes, III

7. LOCATION OF PROJECT
Framingham, Massachusetts

8. DATE PROJECT INITIATED
January 1950

9. OBJECTIVES
To determine those factors which are related to the development or progression of hypertension, hypertensive heart disease and arteriosclerotic coronary heart disease.

10. BACKGROUND
A population sample was drawn in 1950 consisting of 2/3 of the citizens of the Town of Framingham age 30 through 59. Of the selected 6,454 persons it was possible to obtain the cooperation of 4,469. An additional group of 740 volunteers in the same age range was added to the study. All cooperating persons were examined initially and every two years to determine the incidence of new coronary heart disease and hypertensive disease.

The attached table shows the status of examinations as of October 31, 1958.

Technical Services - Epidemiology Section
Framingham, Massachusetts

STATUS OF EXAMINATIONS AS OF OCTOBER 31, 1958

Number selected		6,510
Number examined		4,469 = 68.6%
Plus SX		740
	Total examined	5,209

Number Examined Each Round

Exam. No.	Total	Sample	Sx
I	5,209	4,469	740
II	4,792	4,052	740
III	4,653	3,935	718
IV	4,541	3,843	702
V	2,545	1,876	669
VI	120	71	49

Number Not Examined, Rounds II, III and IV

Round II	417	417	0	
	48	48	-	Deceased
	175	175	-	Exam. later rd
	194	194	-	Lost
Round III	556	534	22	
	100	95	5	Deceased
	138	130	8	Exam. later rd
	318	309	9	Lost
Round IV	668	627	41	
	158	139	19	Deceased
	57	52	5	Exam. later rd
	453	436	17	Lost

Study of the population have been completed to determine occurrence of new cases of disease. Further inquiry was carried on into the population characteristics regarding diet, blood cholesterol values, smoking habits, physical activity and other pertinent factors. The fourth examination of the entire population was completed in July 1958 permitting presentation of this data at the International Cardiology Congress in Brussels, Belgium, September 1958.

Several other presentations of the Framingham data have been presented by Staff members during the year at local and national meetings.

The Study cooperated with the Atomic Bomb Casualty Commission Study in Hiroshima, Japan, assisting the Study to set up some comparable observation on heart disease in a Japanese population.

A Japanese physician from Hiroshima was given three months' training at Framingham to help him set up their cardiovascular study on his return to Japan.

The Study also cooperated with the U.S. Public Health Service Hospital Division and the District Director, Public Health Service to provide a week's training for each of five interns at the Staten Island, Public Health Service Hospital. It is believed that such training was useful in showing potential career officers a connection between clinical medicine and public health.

The Director of the Study was loaned to the World Health Organization for a two month period to assist in the preparation for the convening of an Expert Committee meeting on Cardiovascular Disease held in Geneva, Switzerland. The report of the committee is concerned with the problem of criteria for use in epidemiologic studies of cardiovascular disease.

Assistance was given Dr. Whaley in Anchorage, Alaska in making electrocardiographic readings in his Eskimo Study comparable with Framingham reading.

Plans were prepared to study the population for rheumatoid arthritis as a factor contributing to early onset of degenerative cardiovascular disease in cooperation with Dr. Sidney Cobb of the University of Pittsburgh, School of Public Health and Dr. Arthur Hall of the Robert Breck Brigham Hospital, Boston, Massachusetts. The study was finally begun in December, 1958.

Plans were made with the Albany Group to compare the methods of dietary assessment used in the two studies, to begin early in 1959.

Publications from this project during the calendar year 1958 are as follows:

1. "An Epidemiologic Study of Heart Disease - The Framingham Study". T.R. Dawber, M.D. and W.B. Kannel, M.D. Nutrition Reviews, Jan. 1958.

2. "The Electrocardiogram in Neurocirculatory Asthenia. (Anxiety, Neurosis or Neurasthenia): A Study of 202 Neurocirculatory Asthenia Patients and 757 Healthy Controls in the Framingham Study". W.B. Kannel, M.D., T.R. Dawber, M.D., F.A.C.P., and Mandel E. Cohen, M.D. Ann. Int. Med., Dec. 1958

3. "Blood Pressure and Its Relation to Coronary Heart Disease in the Framingham Study". Abraham Kagan, M.D., Tavia Gordon, William B. Kannel, and Thomas R. Dawber, M.D. To be published.

Department of Health, Education, and Welfare
Public Health Service
Bureau of State Services

Serial No. 6

II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1958

DATE: August 1, 1958

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Prog

3. SECTION
Operational Research

4. DIVISION PROJECT NUMBER
SHS-HD-8

5. PROJECT TITLE
Los Angeles City Civil Service Employees cardiovascular disease study.

6. INVESTIGATORS
Dr. Bruce Picken
Mr. Philip Enterline

7. LOCATION OF PROJECT
Los Angeles, California

8. STATUS
Completed

9. PARTICIPATING ORGANIZATIONS
California State Health Department - facilities
Los Angeles City Health Department - personnel and facilities
University of Southern California - personnel and facilities

10. PRINCIPAL RESULTS

A medical officer assigned by the Control Program continued to work on this project during the past fiscal year. A paper on hypertension was completed. This revealed that about 12% of the Los Angeles Civil Service employees included in the study had hypertension, of which a third had heart disease. Subsequent to the first examination mortality among hypertensive white males diagnosed as having normal hearts was greater than among the normotensives in the 40-54 age group but this difference did not hold under 40 or in the age group 55 and over. It was also observed that hypertensives in the age group 40-54 on the initial examination developed coronary heart disease at a greater rate than normotensives but that this held to a much lesser extent in the age groups under 40 and 55 and over. The difference in prognosis for the hypertensives in the age group 40-54 as compared to other age groups is interesting and will be checked on by further follow-up.

A paper was also prepared on the relationship of physical exertion to the development of coronary heart disease and it was concluded that there was no significant relationship between implied job exertion and subsequent mortality or the development of coronary heart disease. This seems to contradict the generally held belief that physically active persons are at less risk of developing [illegible] disease than more sedentary persons.

Department of Health, Education, and Welfare
Public Health Service
Bureau of State Services

Serial No. 7

II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1958

DATE: August 1, 1958

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Program

3. SECTION
Operational Research

4. DIVISION PROJECT NUMBER
SHS-HD-9

5. PROJECT TITLE
Study of heart sound recorder in detection of heart disease.

6. INVESTIGATORS
Dr. Carl Marienfeld
Dr. Arthur Rikli
Mr. Philip Enterline

7. LOCATION OF PROJECT
Chicago, Illinois

8. STATUS
Completed

9. PARTICIPATING ORGANIZATIONS
Chicago Heart Association - personnel, facilities and funds.

10. PRINCIPAL RESULTS

Further analyses indicates that the heart sound recorder equipment is just as satisfactory as the stethoscope in detecting children with heart disease. These conclusions are based primarily upon the repeated examination of 100 institutionalized children, but also as reported upon last year, to some extent upon the examination of 500 children in another institution. Listening to recorded sounds with either a loud-speaker or earphones was just as effective, as measured by both sensitivity and specificity, as was listening through the stethoscope, in picking out individuals who were later determined in clinical conference of three cardiologists, to have heart disease.

Listening to the recorded heart sounds by earphones was almost as satisfactory as the stethoscope in detecting those cases classified in the clinical conference as having functional murmurs. A number of methodological problems were recognized, and some progress made toward solution.

Department of Health, Education, and Welfare
Public Health Service
Bureau of State Services

Serial No. 8

II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1958

DATE: August 1, 1958

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Program

3. SECTION
Operational Research

4. DIVISION PROJECT NUMBER
SHS-HD-10

5. PROJECT TITLE
Nutrition Study of Seventh-day Adventists at Washington Sanitarium

6. INVESTIGATORS
Carl J. Marienfeld, M.D.
Leonard Syme, Ph.D.
Miss Marjorie Cantoni

7. LOCATION OF PROJECT
Washington, D. C.

8. STATUS
Continuing

9. PARTICIPATING ORGANIZATIONS
Washington Sanitarium and Hospital, Takoma Park, Md. - facilities

10. PRINCIPAL RESULTS

The plan of study was revised considerably during the year. All male Seventh-day Adventist patients, ages 20-64, excluding neuro-psychiatric patients will be studied and matched with controls of the same age group from Baptist and Methodist admissions. It is expected that the total number of patients interviewed will be about 600.

Interview schedule contains detailed questions on foods eaten during the 4 weeks before hospitalization, sociological data and questions regarding the individual's attitude towards religion. Serum cholesterol levels will be determined on samples of blood drawn on the day of admission and three days later. Additional data will be obtained from the hospital records. The collecting of data will begin August 1958, and continue for one to two years at the rate of 5 to 10 interviews per week. Patients who are selected for the study but are discharged from the hospital before being interviewed, will be visited and interviewed at home.

Department of Health, Education, and Welfare
Public Health Service
Bureau of State Services

Serial No 9

II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1958

DATE: August 1, 1958

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Program

3. SECTION
Operational Research

4. DIVISION PROJECT NUMBER
SHS-HD-11

5. PROJECT TITLE
The disabling consequences of coronary heart disease: Nature and causes.

6. INVESTIGATORS
Herbert S. Caron, Ph.D.

7. LOCATION OF PROJECT
Washington, D. C.

8. STATUS
Continuing

9. PARTICIPATING ORGANIZATIONS
D. C. General Hospital - facilities
Georgetown University Hospital - facilities
The Washington Medical Center
Mt. Alto V.A. Hospital
Freedman's Hospital

10. PRINCIPAL RESULTS

Of the 21 male patients, 19 were re-contacted and interviewed. Estimates of "excessive disability" were made on each. On the basis of these ratings, a short quantitative paper was written, describing the relationship between early depression following illness and later excessive disability. In brief, patients who fail to manifest early depression and who deny (or minimize) their symptoms, show the highest rate of later excessive disability.

An objective phase of this study was initiated as a masters dissertation in the Department of Psychology at George Washington University. Forty patients have been contacted to date, their early reactions to the illness have been assessed by questionnaires and rating scales. This project is scheduled for completion this fall.

Department of Health, Education, and Welfare
Public Health Service
Bureau of State Services

Serial No. 10

II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1958

DATE: August 1, 1958

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Pr[illegible]

3. SECTION
Operational Research

4. DIVISION PROJECT NUMBER
SHS-HD-12

5. PROJECT TITLE
Study of public health nursing services to cardiovascular disease patients in Memphis-Shelby County, Tennessee.

6. INVESTIGATORS
Miss Margaret Denham
Mr. Sidney Abraham

7. LOCATION OF PROJECT
Memphis-Shelby County, Tenn.

8. STATUS
Completed

9. PARTICIPATING ORGANIZATIONS
Visiting Nurses Association - facilities
Memphis-Shelby County Health Department - personnel, facilities and funds.

10. PRINCIPAL RESULTS

Paper was presented at Southern American Public Health Association and is being prepared for publication in Public Health Reports. The study showed that nurses employed by tax-supported and private agencies perform many services specifically related to the diagnosis of cardiovascular diseases. It also shows that when frequent visits for the purpose of giving medication are not required, the generalized public health nurse in line with her usual activities performs services significant to the welfare of the patient with cardiovascular disease.

Slides are being prepared on the basis of the study results to give nursing administrators and educators some guide lines for preparing nurses to serve cardiovascular disease patients at home. The slides will, also, serve as a basis for developing a continuing plan for staff education in cardiovascular disease control.

Department of Health, Education, and Welfare
Public Health Service
Bureau of State Services

Serial No. 11

II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1958

DATE: August 1, 1958

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Program

3. SECTION
Operation Research

4. DIVISION PROJECT NUMBER
SHS-HD-13

5. PROJECT TITLE
Familial patterns of serum cholesterol among Vicos Indians of Peru

6. INVESTIGATORS
Dr. Marshall Newman, Smithsonian Institution
Dr. William J. Zukel
Mr. Sidney Abraham

7. LOCATION OF PROJECT
Hacienda Vicos, Peru

8. STATUS
Completed

9. PARTICIPATING ORGANIZATIONS
Framingham Epidemiology Study, National Heart Institute
Smithsonian Institution
Harvard University (Children's Hospital)
Cornell University
(This was a Cornell project from which data relating to cholesterol levels was abstracted. Hence, funds, personnel and facilities were provided by Cornell University and personnel by the other cooperating groups.)

10. PRINCIPAL RESULTS

Draft of paper has been completed; paper is part of a larger report scheduled for publication in Viking Fund Publication in Anthropology. The findings of a low serum cholesterol in a reported low fat intake population supports the hypothesis relating amount of fat diet and serum cholesterol level in populations. Other factors which may be responsible for the low serum cholesterol include: under nutrition abetted by intestinal parasites, an almost wholly vegetarian diet, a tendency for the population not to increase in weight with age, phhsical activity not diminished with age, and blood pressure levels far short of blood pressure levels found in the United States.

Serial No. 12

II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1958

DATE: August 1, 1958

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Program

3. SECTION
Operational Research

4. DIVISION PROJECT NUMBER
SHS-HD-14

5. PROJECT TITLE
Trends in prevalence of rheumatic heart disease among college students.

6. INVESTIGATORS
Dr. Arnold L. Nielsen
Dr. Douglass Thompson, American College Health Association
Mr. Herbert I. Sauer
Miss Margaret Evans

7. LOCATION OF PROJECT
Washington, D. C.

8. STATUS
Continuing

9. PARTICIPATING ORGANIZATIONS
American College Health Association - Student Health Services of 110 participating colleges - personnel and facilities

10. PRINCIPAL RESULTS

During the first year (school year 1956-57), information was reported on 82,820 entering freshmen by 84 colleges and universities. Analysis of these data revealed that there were 1,272 students having either definite rheumatic heart disease or history of rheumatic fever with no heart disease, for a rate of 15.4 per 1,000 students examined. All of these would be considered as candidates for the prophylactic medical regimens indicated in rheumatic fever; however, only about 10 percent, or 128 students are currently following a program of anti-streptococcal prophylaxis.

Thus far for the second year of the study, 76,780 examinations have been reported. An analysis of these revealed that there were 1,187 students with definite rheumatic heart disease or histories of rheumatic fever, for a rate of 15.5 per 1,000 students examined. Of these 156, or 13.1 percent, are currently following a program of prophylaxis.

A paper on the use of prophylaxis in the prevention of recurrent rheumatic fever is being prepared for publication later in the year. A second paper showing the comparison of the two years' results is also planned. A report of the project for the first two years is to be made at the annual meeting of the American College Health Association in Philadelphia in April, 1959.

Department of Health, Education, and Welfare
Public Health Service
Bureau of State Services

Serial No. 13

II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1958 DATE: August 1, 1958

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Program

3. SECTION
Operational Research

4. DIVISION PROJECT NUMBER
SHS-HD-15

5. PROJECT TITLE
Social consequences of heart disease.

6. INVESTIGATORS
Dr. Herbert Caron
Miss Eleonor Morris
Dr. Grace Hussey
Mrs. Hilda LaRocca
Miss Ruth Darr
Miss Alice Davis
Mrs. Eloise Worden

7. LOCATION OR PROJECT
Boston, Massachusetts

8. STATUS
Completed

9. PARTICIPATING ORGANIZATIONS
Massachusetts Department of Public Health
(and six cardiac clinics)

10. PRINCIPAL RESULTS

The exploratory study has been completed and is being written up by the State Health Department. A list of related research questions for further study has been compiled and is being used in consultation regarding studies. Methodological problems of such a cooperative study have been identified. The exploratory schedule has been used in developing study schedules in Pittsburgh and Connecticut.

Department of Health, Education, and Welfare
Public Health Service
Bureau of State Services

Serial No. 14

II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1958

DATE: August 1, 1958

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Program

3. SECTION
Operational Research

4. DIVISION PROJECT NUMBER
SHS-HD-16

5. PROJECT TITLE
North Dakota Coronary Disease Study

6. INVESTIGATORS
Dr. William J. Zukel
Dr. Robert H. Lewis
Mr. Philip Enterline
Mrs. Jean Pekover

7. LOCATION OF PROJECT
Grand Forks, North Dakota
and six contiguous counties

8. STATUS
Continuing

9. PARTICIPATING ORGANIZATIONS
North Dakota State Health Department
North Dakota Heart Association
Grand Forks and Devils Lake Medical Societies, North Dakota

10. PRINCIPAL RESULTS

Analysis of detailed interviews obtained on a census taken in November 1956 was largely completed during the fiscal year. Also, reporting of coronary disease by private physicians was completed on December 1, 1957. A total of 230 cases of coronary disease was reported from the population of 20,000 males 35 years of age and over which is being studied. Of these, 38% were considered as manifest by myocardial infarction while 25% were manifest by sudden death probably due to coronary occlusion. A preliminary tabulation of the incidence of infarction among farmers and non-farmers shows differences of considerable magnitude. A paper reporting preliminary findings and a paper on methodology will be presented at the American Public Health Association meeting this fall.

Department of Health, Education, and Welfare
Public Health Service
Bureau of State Services

Serial No. 15

II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR; 1958 DATE: August 1, 1958

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Program

3. SECTION
Operational Research

4. DIVISION PROJECT NUMBER
SHS-HD-17

5. PROJECT TITLE
Relationship between obesity in childhood and obesity in adult life.

6. INVESTIGATORS
Dr. R. C. Arnold
Mr. Sidney Abraham
Dr. S. Leonard Syme
Miss Marie Nordsieck

7. LOCATION OFPROJECT
Hagerstown, Maryland

8. STATUS
Continuing

9. PARTICIPATING ORGANIZATIONS
Washington County Health Department
Division of Public Health Methods

10. PRINCIPAL RESULTS
A list containing the names of the 100 most overweight and 100 most average-weight children was drawn from the records of the 2,400 physical examinations completed in 1938. Interviews were completed on June 27, 1957 on these two hundred adults. Data from these interviews are now being processed and analyzed.

Department of Health, Education, and Welfare
Public Health Service
Bureau of State Services

Serial No. 16

II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1958 DATE: August 1, 1958

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Program

3. SECTION
Operational Research

4. DIVISION PROJECT NUMBER
SHS-HD-18

5. PROJECT TITLE
Overweight vs. Obesity as related to cardiovascular disease.

6. INVESTIGATORS
Dr. Arnold Nielsen
Dr. H. A. Tyroller
Mr. Sidney Abraham

7. LOCATION OF PROJECT
Asheville, North Carolina

8. STATUS
Continuing

9. PARTICIPATING ORGANIZATIONS
Asheville Research Foundation

10. PRINCIPAL RESULTS

Criteria for new cases of heart disease were prepared by Dr. Nielsen and Dr. Tyroller.

The Asheville Laboratory standardized serum cholesterol determination by exchange of serum samples with the Framingham Laboratory.

I.B.M. cards showing results of first year examination were prepared. (N = 1,932 men)

Results of second examination on Canton plant were sent to this office. Complete follow-up was done on 1,589 or 82.2 percent of those workers originally examined. Follow-up status of the remainder, 343 men, is now being done by Dr. Tyroller. This information pertains to reason for no examination such as: refusal of examination, termination of job, absence on day of examination (will be called for examination), retirement, disability and death.

Department of Health, Education, and Welfare
Public Health Service
Bureau of State Services

Serial No. 17

II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1958 DATE: August 1, 1958

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Program

3. SECTION
Operational Research

4. DIVISION PROJECT NUMBER
SHS-HD-19

5. PROJECT TITLE
Cardiovascular Mortality by Geographic and Related factors, particularly by Country and State of Birth.

6. INVESTIGATORS
Mr. Philip Enterline
Mr. Herbert I. Sauer

7. LOCATION OF PROJECT
Washington, D. C.

8. STATUS
Continuing

9. PARTICIPATING ORGANIZATIONS
National Office of Vital Statistics
National Institutes of Health - Statistical Processing Section

10. PRINCIPAL RESULTS

Special tabulations of arteriosclerotic (including coronary) heart disease deaths for 1950 were made for foreign-born and native-born whites, age 35 to 74. The age-specific rates were consistently higher for foreign-born women than for those born in the U. S., and were also generally higher than the rates for many of the foreign countries from which the foreign-born have come. On the other hand, native-born white males had rates about the same as for foreign-born whites, and, when studied by geographic regions, there are a number of areas in which the native white males had rates higher than did the foreign-born.

Progress is being made on a study of foreign-born whites by country of birth, for decedents in the Middle Atlantic States for 1950, so that age-specific death rates will be computed for persons born in Italy and each of the other countries from which large numbers of immigrants have come - for major specific causes as well as for all causes. Approximately 64,000 IBM cards have been selected from the total of 1,450,000 decedents in 1950 and work will begin shortly on searching for the specific country of birth from the transcript of the death certificate.

Special tabulations have also been requested of mortality by State of birth, for specific causes.

Department of Health, Education, and Welfare
Public Health Service
Bureau of State Services

Serial No. 18

I. RESEARCH PROJECT DESCRIPTION

DATE: August 1, 1958

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Program

3. SECTION
Operational Research

4. DIVISION PROJECT NUMBER
SHS-HD-20 (Continued)

5. PROJECT TITLE
Twenty-year follow-up of stethographically recorded functional murmurs in children.

6. INVESTIGATORS
Dr. Norman Talley
Dr. Carl J. Wiggenfeld
Dr. Bert Boone
Miss Marie Nordsieck

7. LOCATION OF PROJECT
Hagerstown, Maryland

8. DATE PROJECT INITIATED
April 1958

9. OBJECTIVES

To make a longitudinal evaluation using stethograms, electrocardiograms, and X-rays taken 20 years ago among Hagerstown, Maryland school children. If internists are correct and functional murmurs are significant, they should still be present and associated with other manifestations of heart disease in the group of persons followed. If, on the other hand, the pediatricians are right and functional murmurs are normal for children and adolescents, then the murmurs should have largely disappeared, and not more than 5 cases of heart disease should be observed (based on an expected prevalence of 2%* in the general population with an allowance for sampling variation).

10. BACKGROUND

Many attempts have been made to determine the significance of the loud musical systolic (functional) murmur. Direct studies by means of cardiac catheterizations, angiocardiograms, and electrokymograms on children with these murmurs have all been negative. Despite this internists feel this murmur is significant and pediatricians disregard it as a normal finding.

11. PLAN OF WORK

Read 5,000 stethocardiograms on school children obtained in 1937-[illegible] by Dr. Bert Boone in Hagerstown, Maryland for the specific purpose of identifying approximately 100 cases showing the classical stethographic picture of a functional or innocuous murmur. The murmurs will be graded

*Based on 3,300,000 selective service examinations of men between 18 and 37 years of age; conducted from 1940 through 1956.

Department of Health, Education, and Welfare
Public Health Service
Bureau of State Services

Serial No. 18

II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1958 DATE: August 1, 1958

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Program

3. SECTION
Operational Research

4. DIVISION PROJECT NUMBER
SHS-HD-20

5. PROJECT TITLE
Twenty-year Follow-up of Stethographically Recorded Functional Murmurs in Children.

6. INVESTIGATORS
Dr. Norman Telles
Dr. Carl J. Marienfeld
Dr. Bert Boone
Miss Marie Nordsieck

7. LOCATION OF PROJECT
Hagerstown, Maryland

8. STATUS
Continuing

9. PARTICIPATING ORGANIZATIONS
Washington County Health Department, Maryland

10. PRINCIPAL RESULTS
A total of 158 children with loud but apparently functional murmurs have been selected from among 4,000 children having phonocardiographs taken in 1938. Of these, 100 have been located in Hagerstown; 72 have been given physical examinations and laboratory studies, X-ray, phonocardiogram and electrocardiogram. It is anticipated that the remainder will be examined by the end of July.

11.

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Program

3. SECTION
Program Services

4. PROJECT NUMBER
SHS-HD-21

5. PROJECT TITLE
The Use of an Electronic Computer in a Diagnostic Aid

6. INVESTIGATOR
Dr. Arthur E. Hikli (Co-investigators have not yet been selected.

7. LOCATION OF PROJECT
Washington, D. C., and
location of contractor, to be
decided.

8. DATE PROJECT INITIATED
December 1957

9. OBJECTIVES
To explore how an electronic computer might serve as a diagnostic
aid to a physician.

10. BACKGROUND: 1-10 (See attached.)

11. Plan of Work
The work plan will be divided into three phases: (1) feasibility
study, (2) laboratory-scale analysis, and (3) clinical application. In
fiscal year 1958, this project will be concerned only with the first
phase. In this feasibility phase, the aims of the investigation
are (1) to manually collect discrete information on a large
number of normal and pathological subjects, and (2) to process
this information as the input to a general-purpose digital computer

Department of Health, Education, and Welfare
Public Health Service
Bureau of State Services

Serial No. MEY 20

I. RESEARCH PROJECT DESCRIPTION

DATE: August 1, 1958

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Prog.

3. SECTION
Operational Research

4. DIVISION PROJECT NUMBER
SHS-ND-22

5. PROJECT TITLE
Serum Cholesterol level of American Indians on five reservations in the United States.

6. INVESTIGATOR
Sidney Abraham

7. LOCATION OF PROJECT
Serum cholesterol data from Indian Health Survey

Crow - Montana
Yankton - South Dakota
Acoma - New Mexico
San Carlos - Arizona
Lac Court Oreilles - Wisconsin

8. DATE PROJECT INITIATED
July 1, 1958

9. OBJECTIVES
To see whether the mean level of serum cholesterol of clinically healthy Indians residing on five reservations in the United States is low or high in comparison to the mean levels generally accepted for the U.S. population.

10. BACKGROUND
Epidemiologic interest in mean level of serum cholesterol of American Indians stems from the findings of a recent study of Navajo Indians. These findings show significantly lower serum cholesterol concentration in a sample of Navajos as compared with that of a sample clinic group from the U.S. population; nevertheless, the Navajo dietary fat intake was not much less than that of this general U.S. population. These findings are contrary to the suggested (but not conclusive)

10. BACKGROUND (Continued) 3E5-HE-22

evidence, that high fat intake is reflected in the high concentration of serum cholesterol in the blood and perhaps in the tendency to development of atherosclerosis and related conditions of coronary heart disease. It was theorized from these findings that genetic factors rather than dietary factors were the principal cause of the low serum cholesterol level and low coronary heart disease occurrence among the Navajos.

11. PLAN OF WORK

Serum cholesterol data of Indians were obtained from the Division of Public Health Methods. These Indians were examined in the Indian Health Survey conducted by the Division. Means and standard deviations will describe the level of serum cholesterol of each reservation. Mean levels of Indians will be compared with that of a U.S. population having a high level of serum cholesterol. These data will be obtained from one of the clinics which participated in the Cooperative Study of Lipoprotein and Atherosclerosis.

7/31/58

Form No. ORP-2
Oct. 1957

PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
General Medicine and Experimental Therapeutics Branch

Estimated Obligations for FY 1959

Total:	$1,606,248
Direct:	$ 460,000
Reimbursements:	$1,146,248

1. General Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

Studies on Vasoactive Substances (items not covered in other projects).

Principal Investigator: A. Sjoerdsma, M.D., Ph.D.

Other Investigators: J.R. Crout, M.D., L. Gillespie, Jr., M.D., T.P. Waalkes, M.D., and W. Lovenberg, guest worker; S. Udenfriend, Ph.D., J. Pisano, M.D., Mr. C. R. Creveling, (LCB) and E. Labrosse M.D. (NIMH). Technical: C. Muellenberg, D. Watts and E. Marsh (summer student).

Man Years:
Total: 3.50
Professional: 2.0
Other: 1.50

Patient Days: 2,000

Major Findings:

1. Catecholamine Metabolism: Increased amounts of the m-O-methyl metabolites of norepinephrine (normetanephrine and 3-methoxy-4-hydroxymandelic acid) have been demonstrated by solvent extraction and paper chromatographic techniques in the urine of patients with pheochromocytoma. Since milligram-quantities of these metabolites are present in such urine, compared to microgram-quantities of the parent amine, sensitive and specific assay methods for measuring these substances would be quite useful. Intermittent but intensive investigations in methodology have not been successful. This is discussed further in a project of the L.C.B. Major problems are:(1) lack of a specific assay procedure; (2) excretion of normetanephrine predominately as a conjugate (sulfate probably) requires that acid hydrolysis be done but unfortunately the free form of the compound is unstable under these conditions, and; (3) difficulty of separating the methoxy-acid from other phenolic acids of urine. Since many other laboratories are now working on the methodologic problem, our own efforts in this direction will taper off.

Since O-methylation appears to be an important process in the physiologic inactivation of norepinephrine, we have been interested in studying this process in patients. One approach would be to administer large amounts of catecholamine which would be O-methylated and not be metabolized by other routes such as monoamine oxidase; then measure the O-methyl metabolite in the urine. The effects of various drugs on this conversion and the O-methylating activity of the hypertensive individual would be of interest. Preliminary studies were with isoproterenol (Isoprel) given sublingually (100-180 mg in 12h). The O-methyl metabolite was identified in the urine but is not easily measured. In the first study, the control urine also appeared to contain O-methyl-Isoprel. Subsequently, this finding could not be confirmed.

2. Biogenic Amines in Edible Fruits: Early this year, it was found that the banana contains large amounts of serotonin, norepinephrine and its precursor dopamine. This is important clinically in that ingestion of bananas increases the level of urinary metabolites and may lead to false positive chemical diagnoses of carcinoid and pheochromocytoma. An extensive study of amines in fruits, and of the biochemistry of the banana is in progress. Findings to date are reported elsewhere by L.C.B. Highlights include: (1) findings of serotonin and tryptamine in several plant materials (plums, tomatoes, eggplant, etc.), (2) rapid rise in serotonin content of inner peel of banana with ripening, (3) inhibition of banana darkening by treatment with monoamine oxidase inhibitors, and (4) recent demonstration of large amounts of tyramine in the banana (80 µg/gm pulp)!

3. Monoamine Oxidase (MAO) Inhibition: Since MAO is involved in the metabolism of several natural vasoactive amines, inhibition of this enzyme might be expected to affect cardiovascular function. The hypotensive and anti-angina effects of iproniazid are of interest in this regard. Taking a cue from our findings in bananas, a test was developed for measurement of drug-induced MAO inhibition in man, based on the conversion of orally administered serotonin (20-50 mg) to 5-HIAA. One of the first drugs to be studied, 1-phenyl-2-hydrazinopropane (JB-516 of Lakeside), was found to be a potent MAO inhibitor and concurrently discovered to be a potent antihypertensive agent (see separate project for therapeutic evaluation). Curiously enough, this agent is a potent pressor agent in the dog. Other studies center on the problem of the relationship between MAO inhibition and alterations in blood pressure with a final answer not yet available. None of the accepted cardiovascular drugs have been found to be MAO inhibitors.

Several studies were done which suggest that the site of MAO inhibition measured by the oral serotonin test is in the gut and liver. A method for measuring over-all MAO inhibition became desirable. Recent studies suggest that measurements of increases in urinary tryptamine (separate report) and tyramine may afford the sensitive index required.

4. Newly Discovered Amines in Human Urine: We have developed the principle that as yet undiscovered amines may be found in the urine of patients whose MAO has been inhibited with a drug. Tryptamine was easily found under this circumstance (separate report). Just prior to this writing, following the demonstration of tyramine (p-oH-phenylethylamine) in bananas, it was decided to look for this amine in the urine of a patient treated with JB-516 even though the amine has never been clearly demonstrated in mammals. In the first case studied large amounts (800 µg/day) were found and identified chromatographically in one solvent system. We are confident this finding can be confirmed.

5. Histamine & Related Problems: (Several projects written on this subject last year by Dr. T. P. Waalkes). Projects discontinued with departure of Dr. Waalkes from this Section.

Proposed Course of Project:

1. Study catechol-o-methyl transferase in man. The cooperation of Dr. Sidney Archer (Sterling-Winthrop) has been enlisted to provide a catecholamine compound which will be relatively inactive physiologically, an excellent substrate for the enzyme, not be metabolized by MAO and have a substitution on the nitrogen which will enable easy measurement in the urine of the O-methyl metabolite.

2. Study norepinephrine turnover in normals and hypertensives. This was done previously in pheochromocytoma with dopamine-C^{14} and a similar procedure is considered feasible in the prior states if one uses non-isotopic carrier in the preparative procedure.

3. Evaluate further the relative merits of the serotonin conversion test, tryptamine assays and tyramine assays in measuring MAO inhibition in man. Also, delineate physiologic counterparts of the enzyme inhibition by use of a variety of agents. Implicit in this work is the conclusion that one must consider amines other than serotonin and norepinephrine in attempting to explain the pharmacologic effects of MAO inhibitors.

4. Search for other amines ("decarboxylated amino acids") in urine of MAO-blocked patients.

5. Investigate possible decarboxylase inhibitors for physiologic actions. α-methyl dopa is one such compound.

Part B included Yes No

PHS--NIH
Individual Project Report
Calendar Year 1958

Awards

Dr. Albert Sjoerdsma received the 1958 Theobold Smith Award in Medical Sciences from the American Association for the Advancement of Sciences.

Publications

1. T. P. Waalkes, A. Sjoerdsma, C. R. Creveling, H. Weissbach and S. Udenfriend Serotonin Norepinephrine and Related Compounds in Bananas. Science, 127: 3299, p. 648-650, Mar. 21, 1958.

2. E. V. Evarts, L. Gillespie, Jr., C. T. Fleming, and A. Sjoerdsma, Relative Lack of Pharmacologic Action of 3-methody analogue of Norepinephrine (23945) Proc. Soc. Exptl. Biol. & Med. 1958, v98, 74-76.

3. A. Sjoerdsma, W.M. King, L.C. Leeper, and S. Udenfriend, Demonstration of the 3-methody Analog of Norepinephrine in Man, Science 127: 3303, p. 876, April, 1958.

4. A. Sjoerdsma, Carcinoid Syndrome (Carcinoidosis) - (Cecil-Loeb Textbook of Medicine 10th Edition), In Press.

5. A. Sjoerdsma, Clinical and Laboratory Features of Malignant Carcinoid, A.M.A. Arch. Int. Med. In Press.

6. A. Sjoerdsma, Drugs which Alter the Metabolism of Vasoactive Monoamines, Am. J. Card. In Press.

7. A. Sjoerdsma, L. Gillespie, Jr., and S. Udenfriend, A Simple Method for the Measurement of Monoamine Oxidase Inhibition in Man. The Lancet, v2: 7038, p. 159, Jul. 19, 1958.

8. A. Sjoerdsma, Selected Observations on Carcinoid, Mastocytoma and Pheochromocytoma. N.Y. StateJof Med., In Press.

9. A.Sjoerdsma, L. C. Leeper, L.L. Terry, Studies on the Biogenesis and Metabolism of Norepinephrine in Patients with Pheochromocytoma. J. Clin. Inves. In Press.

10. A. Sjoerdsma, Method for Measuring Monoamine Oxidase Inhibition in Man; Application to Studies on Hypertension. Paper for Symposium on Amine Oxidase Inhibitors. N.Y. Acad. Sciences, Nov. 21-24, 1958, To be published.

11. A. Sjoerdsma, Monoamine Oxidase Inhibitors and Related Compounds. Hahnemann Symposium on Hypertension. Dec. 1958, In preparation.

12. A. Sjoerdsma, Catecholamine Metabolism in Patients with Pheochromocytoma. Pharm. Rev. In Press.

1. [illegible]al Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS--NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

Action and Metabolism of Drugs.

Principal Investigator: Louis Gillespie, Jr., M.D.

Other Investigators: Luther L. Terry, M.D., and Albert Sjoerdsma, M.D., Ph.D.

Man Years:
Total: 1.3
Professional: 0.8
Other: 0.5

Patient Days: 4000

Major Findings:

1. Drug Therapy of Hypertension. During the past year we have undertaken the assessment of the antihypertensive effects in man of a yet expanding group of chemical agents which are classified as monoamine oxidase inhibitors. Our studies have been almost exclusively confined to patients with hypertension so that concomitant with biochemical determination of the degree of monoamine oxidase (MAO) inhibition with these various agents, which is described in another project description, we might also determine any possible beneficial effects on blood pressure. To date we have completed or are in the process of evaluating the following drugs: iproniazid, (Marsilid-Hoffman LaRoche), 1-phenyl-2-hydrazinopropane (JB516 of Lakeside), 2 of the Harmala alkaloids - Harmaline and Harmine, Orthoxine (Upjohn), Imipramine (Geigy), Nialamid (Pfizer), and W-1544 (Warner Lambert).

JB516. Although orthostatic hypotensive effects were observed to some extent with several of the above-mentioned agents, it readily became apparent that a reproducible and sustained lowering of the standing blood pressure could be obtained with one of these inhibitors, namely, JB516 Consequently, we extended our studies with this drug to 10 hospitalized patients, demonstrating in 9 a sustained orthostatic lowering of the blood pressure. Each patient was carefully followed clinically as well as from a laboratory standpoint for toxicity. As soon as it was

deemed safe, limited outpatient studies were started, and to date 12 patients have been followed in the clinic for periods of from 2 to 7 months on JB516. This outpatient study has demonstrated that severe hypertension can be managed with JB516 alone or in combination with Chlorothiazide. Again laboratory evidence of toxicity has been absent, but an interesting loss of color discrimination in the red-green spectrum has been observed in 6 of the 12 outpatients, all at maximum doses of JB516. This phenomenon is readily reversible upon discontinuation of the drug. Currently, we have restarted several of this latter group on lower doses of JB516 and are working closely with the ophthalmologists in an attempt to describe this effect in a more objective fashion. At present we are not engaging in further inpatient evaluation of this drug, but we are using the drug as a research tool and as a substitute for ganglionic blocking agents in outpatients. One of the desirable aspects of the use of this agent is the lack of parasympatholytic side effects normally seen when a "ganglionic blocking" drug is employed.

Also during the past year we have arrived at a working arrangement with the Hypertension Clinic at George Washington Hospital. This arrangement allows us to admit patients from their clinic for inpatient study at the Heart Institute. Reciprocally, we are cooperating with them on outpatient studied on a larger scale, utilizing any agents developed here at the Heart Institute which have undergone sufficient trial and appear to be promising. During this year, under the direction of Dr. Harold Orvis and Dr. Irene Tamagna, a small but precise outpatient study was carried out to evaluate iproniazid (Marsilid) alone and in combination with chlorothiazide and including periods of placebo therapy. The results of this study were recently presented to the Washington, D. C. Chapter of the American Federation for Clinical Research and are quite promising except for the dampening effects of liver toxicity reported in recent months and resulting in limitation of the total daily dosage.

Currently, a larger and longer term study has been launched at the same clinic to evaluate further the effectiveness of JB516 in the treatment of hypertension over an extended period. No results from this study are as yet available.

2. Drug Therapy of Angina Pectoris. Our attention was drawn to several early reports that iproniazid was effective in angina. Because of our interest in this drug and the possibility that MAO inhibition might be related to this therapeutic effect mechanistically, we launched a pilot outpatient study early this year, collecting 10 angina patients, largely from a group of hypercholesterolemic patients already being observed at the Heart Institute. These patients were followed for a 2 to 5 month period and the therapeutic effects were recorded. The results were sufficiently promising to lead us to set up a joint double-blind study in the outpatient clinic at D. C. General Hospital - 2 months on the drug

2 months on placebo. The results of this study have been discouraging. Changes in the medical personnel as well as a smaller-than-expected and inconsistent patient population diminished the effectiveness of the study. The results, however, do include a number of cases of good response during placebo administration as well as failure to respond while on the drug. At this point it should also be said that pilot investigation on both an inpatient and outpatient basis has been carried out to determine any possible antianginal effects resulting from JB516 administration. To date, studies performed on 3 outpatients and 3 inpatients fail to show any significant therapeutic effect.

3. Miscellaneous Observations.

a. Effects of MAO inhibition on platelet serotonin levels in man.

Studies were performed in 5 patients. Serial blood samples were drawn during a control period and then during a period of iproniazid administration. A significant rise in the serotonin levels was noted in all patients during iproniazid administration, confirming in man similar results obtained in animals.

b. Norepinephrine infusions.

A number of hypertensive patients were infused with norepinephrine at a constant rate and dosage to determine the standard physiologic (pulse and blood pressure) response in the untreated state. Presently, similar infusions are planned in normotensive individuals. These infusions will serve as control values for subsequent studies in normotensive and hypertensive individuals who have been treated with MAO inhibitors.

c. Extensive studies have been carried out in cooperation with Dr. Donald Gann of the Endocrinology Section on a patient with idiopathic postural hypotension. Physiologic studies were performed which enabled us to localize the lesion to the sympathetic ganglion and characterize to some degree the biochemical deficiency. Furthermore, a substance has been found in this patient's serum which inhibits nerve transmission in a frog nerve-muscle preparation and perhaps may be related to the etiology of this patient's syndrome. Studies are now in progress to attempt to further characterize this substance.

d. In collaboration with Dr. John Gill of the Endocrinology Section we have studied another patient extensively in an attempt to elucidate under what condition chlorothiazide (Diuril) will cause a marked hypotensive response. The results were not entirely conclusive, but suggested that the degree of hypotension produced related directly to the amount of potassium lost in the urine occurring during bicarbonate infusion.

e. One of the MAO inhibitors under study during the past year has been a Harmala alkaloid - Harmine. This compound is unique in that it is the only so-called "reversible" MAO inhibitor currently under study. Current studies being done in collaboration with Dr. Sid Hess of the Clinical Biochemistry Section are designed to measure the blood levels of this compound following both oral and intravenous administration.

Proposed Course of Project:

We are continuing our examination of various MAO inhibitors for their physiologic effects in humans. Continuation of the out-patient studies concerned with JB516 as an antihypertensive agent will be carried on both at the Heart Institute and at the George Washington Hypertension Clinic.

Part B included: Yes

PHS--NIH
Individual Project Report
Calendar Year 1958

Part B

Publications:

1. Shore, P. A., Gillespie, L., Jr., Spector, S. and Prockop, D. Increase in blood serotonin levels induced by iproniazid in man and rabbits. Naturwissenschaften 45: 340, 1958.

2. Gillespie, L., Terry, L. L., Sjoerdsma, A. A new antihypertensive agent, 1-phenyl-2-hydrazinopropane. Abstract and presentation at the meeting of the American Heart Association in San Francisco, Oct. 26, 1958.

3. Gillespie, Louis, Jr., Clinical toxicity of JB516 administration. Presented at Monoamine Oxidase Symposium, New York Academy of Sciences, Nov. 21, 1958. To be published.

4. Axelrod, J., Shofer, R., King, J. K. and Sjoerdsma, A. The fate of papaverine. J. Pharm. & Exptl. Therap. 124:8-15, 1958.

Serial No. NHI-23
1. General Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

Studies of the Cardiovascular Effects of Biogenic Amines in the Dog.

Principal Investigator: Goldberg, L. I., M.D., Ph.D.

Other Investigators: Sjoerdsma, A., M.D., Ph.D., Muellenberg, C., Technician

Man Years:
Total: 0.30
Professional: 0.25
Other: 0.05

Patient Days: None

Major Findings:

1. Cardiovascular Effects of Dopamine, Tryptamine, Serotonin and Norepinephrine: The relative inotropic actions of dopamine, tryptamine, serotonin and norepinephrine were determined in the anesthetized dog by the strain gauge arch technic of Walton and Brodie (Proc. Soc. Exper. Biol. and Med. 1953). The effects of norepinephrine and serotonin have been extensively studied by many investigators, but there have been no reports of the actions of dopamine and tryptamine on the intact dog heart. In view of the recent findings that these latter two amines are present in the normal individual, it appeared important to carefully quantify their actions.

Results of the present investigation have shown that dopamine has a positive inotropic effect at a dose approximately 40 times that of norepinephrine. The pattern of the inotropic response to dopamine is different from norepinephrine in that the peak effect is reached more gradually and lasts about twice as long. The blood pressure response is also different. Norepinephrine produces a brief rise in both systolic and diastolic pressure. Dopamine, on the other hand, produces a biphasic effect with an initial brief (50 seconds) rise in pressure, followed by a more prolonged slight depressor effect, particularly on the diastolic pressure. Tryptamine has been found to have a positive inotropic effect similar to that of serotonin, but equivalent effects require 10 times the dose. Differences in the patterns of response to these amines make it possible to distinguish them in most experiments, but the difference was not as marked as with norepinephrine and dopamine.

2. Effects of Monamine Oxidase (MAO) Inhibitors: The effects of the newer, potent, amine oxidase inhibitors on the inotropic and pressor actions of the above amines have been determined. Four inhibitors of different chemical structure and pharmacological action were used: JB-516 (1-methyl-2-phenyl-ethyl hydrazine HCl), JB-835 (4-phenyl-2-butyl-hydrazine), RO-50700 (2-benzyl-1-picolinylhydrazine) and two Harmala alkaloids, harmine and harmaline. JB-516 was found to exert pronounced inotropic and pressor effects. JB-835, produced similar but reduced effects. RO-50700, however, had little or no cardiovascular action. The Harmala alkaloids, as have been previously reported (Gunn, 1935, Arch. Int. Pharm.) produce an extreme bradycardia.

Sixteen experiments were performed in which the inotropic effects of dopamine, norepinephrine, tryptamine and serotonin were determined by repeated injections. Then the inhibitor was given and the series of amines was again injected. Similar changes in the amine responses were produced by each of the inhibitors. The inotropic effects of tryptamine and dopamine were markedly prolonged (5 to 10 times) and in most experiments the peak effect was potentiated. In several experiments, the pressor effects were also prolonged and potentiated, but were more difficult to quantitate because of their biphasic nature. The actions of norepinephrine and serotonin, on the other hand, were neither potentiated nor prolonged.

Lung or liver biopsies of several of the dogs were obtained before, during, and after inhibition and revealed that the doses of the inhibitors used were adequate to produce complete MAO inhibition.

The results of the present study are the first demonstrations of prolongation and potentiation of dopamine and tryptamine by monamine oxidase inhibitors in the intact dog heart. Additionally, these experiments have shown that the actions of norepinephrine and serotonin are not enhanced under identical conditions. In view of these results, it is important to reassess the concept that the actions of monamine oxidase inhibitors are related primarily to their effects on serotonin and norepinephrine metabolism; it is possible that dopamine and tryptamine have a more important role than previously considered.

3. Effects of Ritalin. Basis for a New Biological Method for Differentiating Dopamine from Norepinephrine: Ritalin has been demonstrated by other investigators (Plummer et al. Schweiz. Med. Woch. 1957) to potentiate the pressor effects of norepinephrine and serotonin. The present study confirms this finding on the contractile force of the dog heart. In addition, it has been found that Ritalin decreases the inotropic responses to dopamine and tryptamine. This has considerable theoretical interest because of structure relationships and receptor response, but also has been found useful as an aid in bioassay. It is extremely difficult at present to differentiate norepinephrine from dopamine by chemical or biological technics. By use of Ritalin and monamine oxidase inhibitors, however, the amounts of dopamine and norepinephrine in a mixture can be

quantified by observing relative potentiation of inotropic effects. This method has already been helpful to Dr. R. Crout and may have more widespread application.

Proposed course of Project:

1. Cardiovascular Effects of Sympathomimetic Amines: Further differentiation of the cardiovascular effects of the naturally occurring sympathetic amines are planned by use of additional technics. In particular, the pulmonary vascular effects of dopamine and tryptamine have never been studied and in view of the pronounced effects produced by serotonin, such studies may be productive.

Additional differentiation of the amines by effects of adrenergic blocking agents such as phenotolamine and dibenzyline are also planned. Such studies, also, have never been done.

2. Monamine Oxidase Inhibitors: The monamine oxidase inhibitors are being studied further in order to establish a physiologic basis for their clinical effects. The action of monamine oxidase inhibitors to produce postural hypotension in the human has not been confirmed by animal studies. It appears unlikely, at present, that such an effect is the result of potentiation of a naturally occurring amine, but this line of investigation is being continued, both in animals and patients. It is therefore important to study the effects of monamine oxidase inhibitors on the sympathetic nervous system by means of ganglionic stimulants and electrical stimulation of sympathetic nerves. Such studies are being planned following both acute and chronic administration of inhibitors in collaboration with Dr. H. Maling, Laboratory of Chemical Pharmacology.

3. Biological Assays: Continuing studies are being conducted to improve existing technics for differentiating sympathetic amines. Collaboration and assistance of investigators having problems requiring such differentiations will be continued.

4. Chemical Structure - Activity Relationships: By using sympathomimetic amines of different chemical structure, it is hoped that additional information will be derived regarding their mode and sites of action. Several new findings have already been demonstrated by the technics presented above, and extension of this work should be fruitful.

Part B included Yes No

PHS--NIH
Individual Project Report
Calendar Year 1958

Part B

Publications

1. Goldberg, L. I., Potentiation of the cardiovascular effects of the Dopamine and Tryptamine by monoamine oxidase inhibitors. N. Y. Acad. of Sciences, Nov. 21-24. To be published.

2. Goldberg, L. I. and Sjoerdsma, A. Cardiovascular effects of naturally occurring sympathetic amines before and after monoamine oxidase inhibition. To be published.

3. Goldberg, L. I. Use of mephenetidin (Ritalin) and monoamine oxidase inhibitors in differentiating norepinephrine from dopamine by bioassay. In preparation.

Serial No. NHI-24
1. General Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

Effects of Dopamine, Tryptamine, and a Monoamine Oxidase Inhibitor in Man: A phase of Studies on Vasoactive Substances.

Principal Investigator: Horwitz, David, M.D.

Other Investigators: Goldberg, L., M.D., Ph.D., Sjoerdsma, A., M.D., Ph.D., National Heart Institute Nursing Service (Technical Assistance).

Man Years:
Total: 0.75
Professional: 0.65
Other: 0.10

Patient Days: 300

Major Findings:

1. Metabolic and Hemodynamic Effects of Dopamine in Man: Dopamine infusions have been made in four patients to date. Uniformly, there has been a rise in systolic blood pressure in the presence of an essentially stable diastolic pressure and pulse rate. Ballistocardiograms reveal a marked increase in the amplitude of the complexes with a shortened Ia - Ja time and steeper Ia - Ja slope. These results are consistent with an increase in cardiac contractile force with little if any peripheral effect. This pattern appears to be unique among the catecholamines.

Effects on blood pressure and pulse become manifest at an infusion rate 30 to 40 times that of norepinephrine and epinephrine administered to the same patients. There have been no arrythmias. The only symptom noted was an increased awareness of the heart beat. A slight increase in the blood glucose level occurred at infusion rates producing cardiovascular effects.

Infusions with tryptamine at rates up to 700 micrograms per minute have been without effect on pulse and blood-pressure.

2. Effect of a Potent Monoamine Oxidase Inhibitor, JB-516, on Angina: Iproniazid, a monoamine oxidase inhibitor, has been reported to be effective in the treatment of angina. This suggested that JB-516, a more potent inhibitor might prove similarly useful. Preliminary observations have been made under controlled conditions in a group of inpatients with advanced angina but conclusions cannot yet be drawn (see project by Gillespie, et al). A liaison has been established with the Baltimore Health Service Hospital and the District of Columbia General Hospital with a view toward utilizing their outpatient angina population for drug studies.

Proposed course of Project:

1. Effects of Dopamine and Tryptamine in Man: Study of the action of the amines on the cardiac function, dynamics of the greater and lesser circulations, carbohydrate metabolism, and oxygen consumption will be pursued.

Modification of these effects in the presence of a potent monoamine oxidase inhibitor and adrenergic blocking drugs will be investigated.

2. JB-516 and Angina: Inpatient studies will be continued and pursued in a larger group of outpatients if it proves promising.

Part B included Yes No

Serial No. NHI-25
1. General Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

Metabolism of Hydroxyproline (OPR)

Principal Investigator: Darwin Prockop, M.D.

Other Investigators: A. Sjoerdsma, M.D., Ph.D., C. Mitoma, Ph.D., and S. Udenfriend, Ph.D.

Man Years:
Total: 0.85
Professional: 0.65
Other: 0.20

Patient Days: 300

Major Findings:

1. From studies over the past two years, Drs. A. Sjoerdsma, J. D. Davidson, Chozo Mitoma and S. Udenfriend have reported an increased urinary excretion of hydroxyproline (OPR) in 8 of 10 patients with Marfan's Syndrome. Since OPR is found only in collagen protein, the high excretion of OPR in Marfan's Syndrome probably reflects a basic defect in the metabolism of collagen.

2. An extension of the initial studies has shown that the daily excretion of OPR is independent of dietary intake. Two patients with Marfan's Syndrome maintained the same excretion on a diet low in OPR and on a normal diet. Feeding OPR alone or in combination with other amino acids increased the free OPR content of urine but did appreciably alter the bound OPR. This effect of orally administered OPR does not have any immediate consequence, since over 95% of urinary OPR is in a peptide-bound form (measurable only after hydrolysis). Feeding OPR in protein form (gelatin), increased both the free and bound OPR in urine. Since equivalent mixtures of amino acids did not affect bound OPR in urine, it appears that OPR peptides can be absorbed from the gastro-intestinal tract and excreted directly. However, large amounts of protein OPR are required to change urinary excretion, and it appears that the normal daily output of bound OPR reflects collagen turnover much as creatinine excretion reflects the turnover of muscle proteins. Up to the present, we have been unable to demonstrate any abnormal metabolism of oral OPR in Marfan's Syndrome.

Proposed Course of Project:

1. The influence of oral amino acids and proteins on urinary OPR will be investigated further in the hope of elucidating the basic defect in Marfan's Syndrome. The fate of intravenously administered OPR will also be studied.

2. An attempt will be made to study the body pool of OPR with isotopically labelled proline and OPR. Preliminary experiments are now underway in animals to see if this approach, as applied for example to the problem of gout, will be feasible in Marfan's Syndrome and other collagen disorders.

3. Animal experiments are being conducted concurrently on the metabolism of OPR and on the effect of various drugs and inhibitors on collagen turnover (see separate project reports by Drs. Mitoma and Udenfriend). Wherever feasible, the results will be applied to clinical problems.

Part B included Yes No

Serial No. NHI-25

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part B:

Publications

1. A. Sjoerdsma, J. D. Davidson, S. Udenfriend and C. Mitoma, Elevated Excretion of Hydroxyproline in Marfan's Syndrome, The Lancet, p. 994, Nov. 8, 1958.

2. C. Mitoma, T. E. Smith, J. D. Davidson, F. M. DaCosta and A. Sjoerdsma, Improvements in Methods for Measuring Hydroxyproline; Application to Human Urine. J. Lab. Clin. Med. In Press.

Serial No. NHI-26
1. General Med. & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

Catecholamine studies, a phase of studies on vasoactive substances.

Principal Investigator: J. Richard Crout, M.D.

Other Investigator: A. Sjoerdsma, M.D., Ph. D.

Man Years:
Total: 1.10
Professional: 0.60
Other: 0.50

Patient Days: 400

Major Findings:

1. Following the demonstration of serotonin and catecholamines in bananas by others in this laboratory, a study of the effect of banana ingestion on the urinary excretion of 5HIAA and catecholamines was undertaken. It was found that 5HIAA excretion increases in an amount approximately equivalent to the mg. of serotonin in the banana pulp eaten. The free catecholamine excretion did not increase, but the acid-hydrolyzable conjugates of norepinephrine and dopamine increased considerably. Large quantities of norepinephrine, dopamine, and DOPA were also given orally to patients without toxic signs and with resultant excretion patterns similar to those found with the banana. This study confirms by an indirect approach the presence of serotonin and catecholamines in the banana and demonstrates the need to collect urine specimens for these assays on a banana-free diet.

2. Work continued on the measurement of catecholamines in blood, though no progress was made in developing methods superior to those now available. Some comments on this work were presented at the Catecholamine Symposium held at the National Institutes of Health on October 16-18, 1958. No further effort in this area is anticipated.

3. We have continued to investigate the usefulness of determining blood catecholamines at various sites within the vena cavae as a diagnostic aid in localizing pheochromocytomas, the blood samples being obtained by venous catheterization. Data is now available from five catheterizations in four patients (two catheterizations at NIH with the assistance of Samuel Fox, M.D., two at the Bethesda

Naval Hospital with the help of Dr. J. Masur, and one at the George Washington University Hospital by Dr. G. Kelser). The tumor was localized in three patients, but not in the fourth. This study is nearing completion and further work in this area is not anticipated.

4. Choline 2, 6-xylyl ether bromide (TM-10) has been reported by others to inhibit catecholamine synthesis in the whole animal. We have confirmed that it causes a decrease in adrenal catecholamines compatible with such an action and are planning a more detailed study of its biochemical effects.

5. The laboratory has continued to perform a service function for outside institutions by determining urinary catecholamines on selected hypertensive patients. An average of 4-5 such assays is performed each week. This function fills a need in the local medical community and helps provide the section with valuable outside contacts for patient material.

Proposed Course of Project:

1. Efforts are being made at present to assay dopamine as well as norepinephrine and epinephrine in urine and tissue. Another area of methodologic interest is the development of a simple colorimetric assay for norepinephrine in urine for diagnostic purposes.

2. We anticipate further study of TM-10 (see Item C-4 above).

3. Study of the O-methylation pathway of catecholamine metabolism as noted in Dr. Sjoerdsma's report.

Part B included Yes No

Serial No. NHI-26

PHS--NIH
Individual Project Report
Calendar Year 1958

Part B:

Publications

1. Crout, J. R., Some spectrophotofluorimetric observations on blood and urine catechol amine assays. Pharm. Rev. In Press.

2. Crout, J.R., Sympathetic and adrenal medullary considerations in hypertension. Hahnemann Symposium on Hypertension. In Press.

Serial No. NHI-27
1. Gen. Med & Exp. Therap.
2. Experimental Therapeutics
3. Bethesda, Md.

PHS--NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

Metabolism of tryptamine and indole acetic acid in man.

Principal Investigator: John A. Oates, M.D.

Other Investigators: A. Sjoerdsma, M.D., Ph. D.
Perola Zaltzman
Sidney Udenfriend, Ph. D. (L.C.B.)

Man Years:
Total: 1.80
Professional: 0.60
Other: 1.20

Patient Days: 300

Project Description

Major Findings:

1. Tryptamine

Studies previously carried out by this group and the Laboratory of Clinical Biochemistry demonstrated the presence of tryptamine in animal tissues, arising from the decarboxylation of tryptophan. Sullivan (1922) and Rodnight (1956) have presented some evidence that tryptamine is present in human urine, but lack of a suitable method has heretofore limited studies of its metabolism in man.

Initially, a method for the analysis of tryptamine was devised which was dependent upon a basic extraction into Benzene and measurement of the color (produced by reaction with Xanthydrol) in a long light path colorimeter. This proved not to be sensitive enough to quantify the small amounts present in urine, but has subsequently had successful application to analysis in animal tissues.

A more sensitive method was devised for urine which utilizes the stable fluorescence of tryptamine at a basic pH. The specificity of the method has been confirmed by repeated scans of fluorescence - activitation pattern of samples, by chromatography in several systems, and by counter current distribution.

Normal daily excretion has been found to range from 25 to 130 micrograms/day.

Tryptamine is a good substrate for the enzyme monoamine oxidase (MAO). When MAO inhibition is produced in man with 1-phenyl-2-hydrazinopropane (JB-516), increases in urinary tryptamine to 4-10 times the control level occurs. To our knowledge this increase exceeds those seen in any other endogenous amine studied in the presence of MAO blockade. Consequently, the measurement of tryptamine excretion is being utilized as an index of MAO inhibitory action of investigational drugs. With this index, information regarding the relative potency, onset of action, duration of effect and the dose response of MAO inhibitors can be obtained.

To date, only preliminary studies on the metabolic pathways of tryptamine are complete. Intravenous infusion of tryptamine in a patient was found to produce a marked increase in urinary IAA but no rise in 5HIAA. This, together with the finding of normal tryptamine excretion in urine of 3 patients with malignant argentaffinoma is presumptive evidence against the formation of 5-hydroxytryptamine (serotonin) from tryptamine. Tryptophan loading in man has been shown to increase tryptamine excretion 2-3 fold. When this is done to patients on JB-516, quite high levels of excretion are obtained and the patients voice subjective complaints usually summarized as a "drunk feeling".

2. Indole Acetic Acid (IAA)

The previous studies of IAA excretion in patients have been extended slightly, with the finding of increased excretion in several gastrointestinal conditions other than sprue, including pancreatic insufficiency and a blind intestinal loop. A study was attempted to confirm the finding of Kral et al (1956) that IAA excretion increases with exercise. It was found that IAA excretion rises after exercise, but a striking diuresis also ensued after exercise. Therefore, the effect of urine flow on the hourly excretion of IAA was studied in normal subjects, and a definite relationship was found to exist; as much as a two-fold increment in IAA output occurred after a water load.

Proposed course of project:

1. Tryptamine

The tryptamine assay will be used in conjunction with the serotonin tolerance test in the clinical evaluation of MAO inhibitors.

The metabolic pathways of tryptamine given orally and IV are to be further studied, and the effect of urine flow on the excretion of such amines determined. Tryptamine assays will be done on the urine of patients with a variety of clinical disorders.

2. IAA

Further studies of IAA and 5HIAA excretion in patients with intestinal diseases are being initiated in cooperation with the New York Hospital and Bellevue's G.I. Clinic.

Part B included Yes No

Serial No. NHI-27

PHS--NIH
Individual Project Report
Calendar Year 1958

Part B.

Publications:

1. Weissbach, Herbert; King, William; Sjoerdsma, Albert and Udenfriend, Sidney. Studies on the biosynthesis of indole-3-acetic acid in animals and a method for its assay. Tryptamine formation by animal tissues and human fecal bacteria. J. Biol. Chem. In Press.

2. Oates, J. and Zaltzman, P. Discussion of measurement of MAO inhibition at "MAO Symposium" of New York Academy of Science, November, 1958. To be published.

Serial No. NH1-28

1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Hemodynamic changes following production of myocardial ischemia.

Principal Investigator: Guy O. Barnett, M.D.

Other Investigators: Samuel M. Fox III, M.D., and Donald L. Fry, M.D.

Cooperating Units: None

Man Years (Calendar Year 1958): .5

Patient Days (Calendar Year 1958): None

Project Description:

Objectives: The hemodynamic changes following production of a generalized myocardial ischemia will be studied in both the open and the closed-chest dog. Techniques will be perfected so that instantaneous aortic diameter can be obtained, thus allowing calculation of instantaneous cardiac output.

After the elucidation of the changes following acute ischemia, the chronic effects of myocardial ischemia on hemodynamics in the unanesthetized animal will be studied.

Methods employed: The left ventricular and aortic pressures will be studied by means of chronic indwelling catheters. The instantaneous aortic velocity will be measured using only local anesthesia for the arterial cut-down. Mean flows will be measured by the indicator dilution technique. Myocardial ischemia will be produced by both gradual occlusion of a single coronary artery and by graded repeated coronary artery plastic bead embolization.

Major Findings: Using the anesthetized, thoracotomized dog, left ventricular myocardial ischemia was acutely produced by temporary coronary artery ligation. Recording was done of the intraventricular pressure, the aortic pressure, the ECG, and the instantaneous ascending aortic blood velocity (as measured by the catheter tip method developed by this Section). The duration of the ligation was limited to between one and four minutes. Over 15 successful ligations were performed in six different animals.

Part B included: Yes X No

Serial No.

1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Pretracheal Approach to Left Heart Catheterization.

Principal Investigator: Samuel M. Fox III, M.D.

Cooperating Units: The assistance and technical facilities of the Operating Room Service and the Clinic of Surgery have been greatly appreciated.

Man Years (Calendar year 1958): .5

Patient Days (Calendar year 1958): 112

Project Description:

Objectives: Continued use is anticipated but because of the potential damage that can occur to the systemic arteries, it appears that this approach is likely to be justified chiefly for clinical physiologic studies where the required premedication needed for the transbronchial or other approaches would produce non-basal conditions.

Methods employed: Over 50 needle passages have been done in cadavers followed by a dissection for anatomic orientation. An original and a modified needle have been made, both of which have been found to work well in eight living human patients. No serious difficulties have occurred with the passage of the needle and increased technical facility and knowledge has been obtained. One serious complication occurred during catheterization of the left heart through the needle, but this was not considered a reflection upon the type of needle approach but upon the handling of the catheter.

Part B included:	Yes	No
		X

Serial No. NHI-30

1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Pathogenesis of Chronic Diffuse Obstructive Pulmonary Emphysema

Principal Investigator: Donald L. Fry, M.D.

Other Investigators: Donald P. Schilder, M.D. and Dali J. Patel, M.D.

Cooperating Units: None

Man Years (Calendar year 1958): .5

Patient Days (Calendar Year 1958): 50

Project Description:

Objectives: Theoretical considerations have been developed that indicate that an abnormal stress distribution within the structure of the lung may be the major factor in the production of the disruptive lesions of emphysema and furthermore that this abnormal stress distribution may result from bronchialar or bronchial abnormalities that may be either congenital or acquired.

Major Findings: Progress during the past six months has been directed toward developing techniques so that the stress distribution over the normal human lung may be studied. To this end, an improved type of intraesophageal pressure measuring system has been developed.

Proposed course of project: Perfection of the instrumentation.

Part B included:	Yes	No
		X

Serial No. NHI-31

1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Significance of the Maximum Expiratory Flow Volume Curve in Human Subjects

Principal Investigator: Robert E. Hyatt, M.D.

Other Investigators: Donald P. Schilder, M.D., and Donald L. Fry, M.D.

Cooperating Units: None

Man Years (Calendar Year 1958): 1

Patient Days (Calendar Year 1958): 400

Project Description:

Objectives: Current research, when resumed, will be directed toward evaluating the relative importance of viscosity and density of the respired gas in determining the shape of the maximum expiratory flow-volume curve. From these studies, it is hoped that sufficient simplification can be achieved in the mathematical structure describing this curve that computer techniques can be applied to these curves to draw inferences as to the bronchial compliance, resting dimensions and viscosity, and the lung parenchymal compliance and viscosity.

Methods employed: The four variables, flow, volume, pressure and time, that describe the mechanical behavior of the human lung were studied in normal, cardiac and emphysematous individuals.

Major findings: The experimental data confirms the theoretical prediction that of all the relationships involved, the relation of the maximum achievable expiratory flow at any degree of lung inflation is a constant one for a given subject, is independent of upper airway resistance, and furthermore that this maximum expiratory flow versus volume curve reflects the physical properties of the intrathoracic pulmonary tissues. This was determined from model studies in which liquid flow through rubber tubes under varying conditions of stress was studied.

Part B included:	Yes	No
		X

Serial No. NHI-32

1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Investigations in Cardiac Anatomy, Descriptive, Developmental and Histochemical.

Principal Investigator: Robert P. Grant, M.D.

Other Investigators: Thomas N. Lynn, Jr.,M.D.

Cooperating Units: None

Man Years (Calendar year 1958): .5

Patient Days (Calendar Year 1958): None

Project Description:

Objectives: 1. A study was completed involving the syndrome of cardiac dextroversion, using a topological approach to the anatomic description. Developmental possibilities were included in the study.

2. A study was started in May 1957, in which specially fixed hearts, both normal and abnormal, are studied in regard to the size and distribution of muscle bundles. It is hoped that this study will lead to an accurate and simplified method of classifying the defects seen in congenital heart disease.

3. A study was begun in April 1958, which was designed to correlate the amount of fibrosis present in the different heart chamber walls with the disease process and electrocardiogram. This is done by a chemical analysis of the myocardial tissue for hydroxyproline. Thus far, equipment has been gathered and a satisfactory method of chemical analysis has been found. The gathering of data has been proceeding slowly because of apparatus breakdowns but it is hoped to continue collecting data until a statistically significant number of cases is reached.

Part B included: Yes X No

Serial No. NHI-32

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publication other than abstracts from this project:

Grant, Robert P., M.D. The Syndrome of Dextroversion of the Heart. Circulation 18:25, 1958

Serial No. NHI-33

1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Basic Physical Characteristics of the Pulmonary Vascular System.

Principal Investigator: Dali J. Patel, M. D.

Other Investigators: Donald L. Fry, M.D., Donald P. Schilder, M.D., and Samuel M. Fox III, M.D.

Cooperating Units: None

Man Years (Calendar year 1958): 1

Patient Days (Calendar Year 1958): 10

Project Description:

Objectives: The simultaneous measurement of blood pressures, flow and velocity have been continuously recorded from the dog's pulmonary vascular system. From these measurements estimates of inertance, capacity and resistance of the system can be computed continously. The effect of intrathoracic pressure, drugs, breathing rate, mitral insufficiency and various other interventions have been studied.

Major Findings: Results to date have been:

(1) The pulmonary flow, blood velocity, PA pressure and the pressure drop across the pulmonary capillaries, all have very similar patterns with time. The peak flow leads the pressure in the arterial system, suggesting a small capacitance and small inertance in the pulmonary artery. In the pulmonary vein both are disassociated and markedly damped, suggesting a very large capacitance in the pulmonary venous bed.

(2) During positive pressure inflation, the vascular resistance increases.

(3) The production of mitral insufficiency produces an easily measured retrograde flow in the pulmonary veins in dogs. It is hoped that this can be modified to be used in human subjects for semi-quantitative estimation of mitral regurgitation. Preliminary studies similar to the above have been started in human subjects with encouraging results.

Proposed course of project: Pursuance of the above studies.

Part B included: Yes No

Serial No. NHI-34

1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Pre and Postoperative Studies in Patients with Increased Pulmonary Vascular Resistance.

Principal Investigator: Samuel M. Fox III, M.D.

Other Investigators: N. P. Collins, M.D., J. Gilbert, M.D., A. G. Morrow, M.D., and E. Braunwald, M.D.

Cooperating Units: All equipment used up until July 1 was that of the Clinic of Surgery. Most of the work will be continued there.

Man Years (Calendar year 1958): 1

Patient Days (Calendar Year 1958): 200+

Project Description:

Objectives: The anticipated direction of this study will be to accumulate more case study experience and to include a correlation with the lung biopsies and clinical and symptomatic changes.

Methods employed: Over two dozen cases with postoperative studies of the pulmonary vascular resistance have had a comparison made with similar studies done preoperatively. Most of these studies have two determinations made at rest and one during the 7th to 10th minute of moderate exercise.

Major Findings: A correlation is made between the type of change and the nature of the lesion and pressure-flow phenomena of each case study.

Part B included: Yes X No

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Effect of Valsalva Maneuver on Pulmonary Vascular Resistance.

Principal Investigator: Donald P. Schilder, M.D.

Other Investigators: Dali Patel, M.D., Samuel Fox III, M.D., and Donald L. Fry, M.D.

Cooperating Units: None

Man Years (Calendar Year 1958): .5

Patient Days (Calendar year 1958): 90 - 3 patients

Project Description:

Objectives: The study entails the measurement of blood flow with indicator dilution technique, the measurement of the intrathoracic pressure with an intraesophageal balloon and the measurement of the pressure drop across the pulmonary capillary bed with a differential pressure recording system during the Valsalva maneuver. The instrumention has been completed.

Major Findings: Initial observations in three subjects, a model, and a dog lung preparation would indicate that the pulmonary vascular resistance increases during the Valsalva maneuver.

Proposed course of project: Additional observations are now being made in order to complete the project. Supportive data obtained with a catheter tip method of measuring pulmonary blood velocity is being collected.

Part B included:	Yes	No
		X

Serial No. NHI-36

1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Study of the measurement of the intrathoracic pressure throughout the chest and its relation to pulmonary stress distribution.

Principal Investigator: Donald P. Schilder, M.D.

Other Investigators: Robert E. Hyatt, M.D., and Donald L. Fry, M.D.

Cooperating Units: None.

Man Years (Calendar year 1958): 1

Patient Days (Calendar year 1958): 140 - 10 patients

Project Description:

Objectives: Human subjects are being studied with small intraesophageal balloons at different chest levels during various respiratory maneuvers in order to estimate the stress distribution throughout the chest of the normal and emphysematous patients.

Major Findings: An improved intraesophageal pressure recording system for the measurement of changes in intrathoracic pressure in man has been developed. A system for measuring intrathoracic pressure variations in the dog chest has been completed.

Preliminary observations would indicate that the intrathoracic pressure varies throughout the chest of the dog.

Part B included: Yes X No

Serial No. NHI-37

1. Cardio pulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Intracardiac Electrocardiography

Principal Investigator: Thomas N. Lynn, Jr., M.D.

Other Investigators: Samuel M. Fox III, M.D., Robert P. Grant, M.D.

Cooperating Units: Some much appreciated support was provided by the Clinic of Surgery, NHI, but their schedule has become too busy to allow further use of their facilities.

Man Years (calendar year 1958): Total: .5

Patient Days (calendar year 1958): 15

Project Description:

Objectives: It is anticipated that studies will be done during Valsalva stress and/or the application of a G-suit to correlate the ECG changes with the pressure-volume characteristics of the two ventricles.

Major Findings: Little progress has been made in this study due to two factors. The first is the apparent inability of commercial and research and development companies to manufacture an adequate small catheter to put within a #17 needle for left heart studies.

The second factor is the setting up of a new catheter laboratory in the Section of Cardiodynamics. This will provide good facilities but the acquiring of equipment and training of personnel has delayed other than three additional intracardiac electrode explorations in the calendar year.

Part B included:	Yes	No
		X

Serial No. NHI-38

1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Pharmacodynamic Studies of the Pulmonary Circulation

Principal Investigator: Samuel M. Fox III, M.D.

Other Investigators: Donald P. Schilder, M.D., Dali J. Patel, M.D., and Donald L. Fry, M.D.

Cooperating Units: Previous to July 1, all the facilities used for the acute studies were those of the Clinic of Surgery, NHI

Man Years (Calendar year 1958): .5

Patient Days (Calendar Year 1958): 80

Project Description:

Objectives: While still on the surgical service, the effects of Acetyl Choline were investigated in 8 cases of precapillary pulmonary hypertension and two more detailed studies with Methoxamine were complete. The latter did not show any significant fall in pulmonary vascular resistance accompanying the rise in systemic pressure.

Major Findings: Progress has been slow due to the transfer of operations to a new laboratory that is being set up in our Section.

Proposed course of project: Continuation will be along much the same line with the later addition of the velocity catheter to the studies.

Part B included:	Yes	No
		X

Serial No. NHI-39

1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies upon the factors influencing the character and timing of the opening snap in mitral stenosis.

Principal Investigator: Samuel M. Fox III, M.D.

Other Investigators: D. Nelson, M.D. and A. G. Morrow, M. D.

Cooperating Units: Equipment from Clinic of Surgery, NHI

Man Years (Calendar year 1958): .5

Patient Days (Calendar year 1958): 20

Project Description:

Objectives: No plans have been made for an early return to this work, but if the pretracheal left heart technique proves applicable, some correlative phonocardiographic studies seem attractive. The same is true of the velocity catheter when it is better established.

Major Findings: Cancelled due to the publication of an apparently adequate similar study by others. Little of definite value had been accomplished due to the need for better phonocardiographic instruments now available in the Clinic of Surgery, NHI.

Part B included:	Yes	No
		X

Serial No. NHI-40

1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies concerning the determination of the instantaneous pulsatile blood velocity.

Principal Investigator: Samuel M. Fox III, M.D.

Other Investigators: Donald L. Fry, M.D., Donald P. Schilder, M.D., Dali Patel, M.D., Theodore Cooper, M.D. and Guy O. Barnett, M.D.

Cooperating Units: None

Man Years (Calendar Year 1958): 1

Patient Days (Calendar Year 1958): 100+

Project Description:

Objectives:

(1) The catheter-computer method for measuring instantaneous blood velocity has been compared simultaneously with electromagnetic flowmeter in dogs. The validity of the catheter technique appears to be confirmed but further more refined studies are being undertaken.

(2) The changes of aortic blood velocity accompanying the acute production of aortic insufficiency in dogs have been studied at various aortic levels.

(3) A new valving mechanism for the estimation of "zero flow" has been designed, built and is now being tested with encouraging results.

Major Findings: Perhaps the greatest progress during the past six months has been the development of techniques for the application of the blood velocity catheter to human subjects. Blood velocity has been measured successfully in seven human subjects with no serious complications. Large differences in velocity patterns have been observed in patients with aortic insufficiency and myocardial disease. The significance of these differences remains to be established.

Page 2 - Studies concerning the determination of the instantaneous pulsatile blood velocity.

Proposed course of Project: Direction of current research on the application of this to human and animal work will be as follows:

(1) Definition of normal velocity characteristics at different levels in the aorta of dogs and man.

(2) Studies of changes that can be produced with various drugs, changes in intrathoracic pressure and various other interventions in dog and man.

(3) Studies of the usefulness of the velocity technique in post-operative evaluation of myocardial function (to be a joint project with the Clinic of Surgery).

Part B included:	Yes	No.
		X

Serial No. NHI-41

1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Clinical and Experimental Electrocardiography.

Principal Investigator: Robert P. Grant, M.D.

Other Investigators: Thomas N. Lynn, Jr., M.D. and Samuel M. Fox, M.D.

Cooperating Units: None

Man Years (Calendar year 1958): .5

Patient Days (Calendar year 1958): 24

Project Description:

Objectives:

(1) A study of the electrocardiographic dissection and characterization of the pre excitation type of the Wolff-Parkinson-White Syndrome has been successfully completed. This study provides a logical explanation for the deformity of the spatial QRS loop seen in this syndrome.

(2) Work was begun in September 1958 on a project designed to elucidate the electrocardiographic changes caused by surgical closure of ostium secundum defects in the human subject. Special emphasis has been given to the change in the duration of the QRS deflection and it has been found that in 21 of 23 cases, there has been a decrease in the duration of the total QRS forces following closure of the defect. This is currently being written up for publication.

(3) A study was begun in June 1958, using electrocardiographic data and autopsy reports from the Clinical Center, Georgetown Hospital, and George Washington Hospital. The purpose of this study is to find better electrocardiographic criteria for the diagnosis of myocardial infarction. It is anticipated that this study will continue for several years in its present form until a number of cases large enough for statistical analysis is compiled.

(4) A study was started in September 1958 in which certain hemodynamic events in the cardiac cycle (right ventricular filling), are modified and the change in the QRS forces noted. This is currently being done in normal patients with right ventricular conduction delays, using the Valsalva and Muller maneuvers to alter right ventricular filling. It is planned to continue the study in its present form and to obtain hemodynamic and electrocardiographic data during balloon occlusion of atrial septal defects.

(5) Two studies have been continued from 1957. The first is an electrocardiographic-anatomic correlation in congenital heart disease utilizing material obtained at the Clinical Center and at the Childrens Hospital of D.C. The electrocardiographic description and analysis employs the vector approach. It is planned to continue this study in its present form. Completion should occur early next year. The other study carried over from last year involves the description of the unusual frontal plane vector loop that occurs in the majority of cases of ostium primum and common A-V canal. Pathologic-electrocardiographic correlations are being attempted. It is planned to continue this study in its present form.

Part B included:	Yes	No
	X	

Serial No. NHI-43

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publication other than abstracts from this project:

Grant, Robert P., Tomlinson, Fred B. and Van Buren, James K. Ventricular Activation in the Pre-Excitation Syndrome (Wolff-Parkinson-White). Circulation 18:355, 1958.

Serial No. NHI-42

1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Development and Evaluation of Basic Instrumentation for Application in the Field of Blood Flow and Pressure Measurement.

Principal Investigator: Donald L. Fry, M.D.

Other Investigators: Samuel M. Fox III, M.D. and Donald P. Schilder, M.D.

Cooperating Units: None

Man Years (Calendar year 1958): 2

Patient Days (Calendar Year 1958): 50

Project Description:

Objectives:

(1) A considerably more sophisticated flow generator for testing and validating the catheter velocity measuring device is being constructed. This system will not only permit more accurate validation of the catheter technique but also will allow a more careful examination of the frequency dependency of the coefficient of friction in the blood flow of large vessels. This factor, unlike the blood inertance, is ill-understood and constitutes one of the weakest of the set of assumptions involved in the theoretical structure describing the catheter-computer blood velocity measuring system.

An even more important parameter of cardiovascular function than blood velocity would be the instantaneous blood flow in a blood vessel. From the above studies it is hoped that information about velocity profiles across the blood vessel will be obtained and from this information knowledge of instantaneous blood velocity will permit the computation of instantaneous blood flow. Since the instantaneous aortic or blood vessel diameter will be necessary in these computations, current research is also directed toward measurement of this parameter.

Page 2 - The Development and evaluation of basic instrumentation for application in the field of blood flow and pressure measurement.

Methods employed: A set of recording calipers of low mechanical impedence that may be attached to the blood vessel for sensing its instantaneous diameter has been developed. The stantaneous diameter will then be correlated with the instantaneous transmural pressure difference at that point.

Major Findings: Preliminary studies suggest that the aorta can be considered as an approximately linear system over physiological ranges; that is, that the pressure tracings with time very closely resembles the diameter tracings with time. Thus, if the blood velocity profile across the vessel is essentially blunt or has a known correction factor, and if in a given individual the proportionality between the pressure and vessel diameter can be established angiographically, then it should be possible to use the aortic blood velocity measuring device for instantaneous aortic blood flow.

Objectives:

(2) Current research with the improved intraesophageal pressure measuring device is directed toward the continuous monitoring of intrathoracic pressure during all of the cardiopulmonary physiologic studies in progress in this laboratory and toward the measurement of regional variations in intrathoracic pressure during a cough and other strenuous ventilatory maneuvers.

Major Findings: The former sheds light on the factors controlling venous return to the thorax and the latter sheds light on the possible role of stress distribution in the lung in the pathogenesis of pulmonary emphysema.

Part B included:	Yes	No
	X	

Serial No. NHI-42

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

1. Fox, Samuel M. III, M.D., Alexander Mallos, B.S., Theodore Cooper, M.D.,Ph.D., Donald L. Fry, M.D. Comparison of the Differential Pressure Catheter-Computer Technique with other Methods for the Measurement of Instantaneous Pulsatile Blood Velocity. The meetings of the American Heart Association, San Francisco, 1958.

2. Fry, Donald L., Noble, F.W. and Mallos, A. J. An Evaluation of Modern Pressure Recording Systems. Circ. Research 5:40, 1957.

3. Fry, Donald L.,M.D., Hyatt, R. E.,M.D., McCall, C. B. and Mallos, A.J. Evaluation of Three Types of Respiratory Flowmeters. J. of Appl. Physiol. 10:210, 1957.

4. Fry, Donald L.,M.D., Noble, F.W. and Mallos, A. J. An Electric Device for Instantaneous and Continuous Computation of Aortic Blood Velocity. Circ. Research 5:75, 1957.

Serial No. NHI-43

1. Cardiopulmonary Physiology
2. Cardiodynamics
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title Studies on Coronary Flow.

Principal Investigator: Samuel M. Fox III, M.D.

Cooperating Units: None

Man Years (Calendar Year 1958): .5

Patient Days (Calendar Year 1958): None

Project Description:

Objectives: Because of the great need for a simple means for determining coronary blood flow, an assessment of the possibility of total coronary sinus extration of blood was undertaken.

Methods employed: Special catheters designed for human use have recently been received and their capacity to fill the requirements are being tested in dogs.

Major Findings: In the dog it was found possible to extract all the blood returning to the coronary sinus for periods up to one minute after balloon occlusion.

Proposed course of project: We will continue with dog testing and if modifications are needed, they will be made before trials in human subjects are attempted.

Part B included	Yes	No
		X

Serial No. NHI-44
1. General Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

Effect of Chronic Hypercalciuria on Renal Concentrating Mechanism and Sodium Conservation.

Principal Investigator: Gill, John R., M.D.

Other Investigators: Bartter, F. C., M.D. and Smith, G.

Man Years
Total: .56
Professional: .23
Other: .33

Patient Days: 200

General Purpose:

1. To study the effect of hypercalciuria, parathyroid hormone, and vitamin D on the ability of the kidney to concentrate.

2. To determine the effect on the concentrating defect of restoring urinary calcium to normal in hypercalciuric patients.

3. To determine whether the concentrating defect is associated with inability to retain sodium.

It has been reported that patients with hypercalcemia and hypercalciuria may lose the ability to concentrate urine even in response to pitressin. The present studies were instigated to determine what factors are essential for the development of this defect.

Major Findings:

Eight patients with hypercalciuria were studied. In all urinary concentrating ability was impaired as judged from the maximum urinary osmolality obtainable with dehydration for 16 hours and pitressin, or from determination of $T_mC\ H_2O$ after the method of Zak, Brun and Smith.

When ethylene diamine tetra acetate (EDTA) was given by mouth to return urinary calcium to normal, there was significant improvement in concentrating ability. Hypervitaminosis D and hyperparathyroidism appeared capable of producing a more severe concentrating defect for the degree of hypercalciuria than was found in sarcoidosis, essential hypercalciuria, etc. All subjects with the defect could conserve sodium on a low sodium intake.

Proposed Course of Project:

To obtain further data relating to effects of vitamin D and parathyroid hormone on water conservation.

Part B included Yes No

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part B:

Publications:

Gill, John R., Jr., and Bartter, Frederic C., Abstract entitled "Effect of Chronic Hypercalciuria on Renal Conservation of Sodium and Water", Clin. Res. Proceedings, January 1959.

Serial No. NHI-45
1. General Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

Structure-function Relationships in Anabolic Steroids

Principal Investigators: Schedl, H., M.D., Ph.D., Bartter, F. C., M.D.

Other Investigators: C. S. Delea, C. P. Kirby, and G. S. Greene

Man Years
Total: 1.10
Professional: .43
Other: .67

Patient Days: 300

General Purpose:

1. To correlate protein-anabolic action of steroids with structure.

2. To determine relative sodium-retaining activity of these steroids.

Major Findings:

The essential analytical data have been obtained to complete the study of the effect of 19-methylation on the activity of steroids with a two carbon side chain in the α- position at C-17. Compounds lacking the 19-methyl group, i.e., the 19-nor compounds (17α-ethyl-, 17α-vinyl-, and 17α-ethinyl-) are as potent as 17-methyl testosterone in causing nitrogen retention in normal young women. The corresponding compounds with the 19-methyl group did not cause nitrogen retention.

Weight gain and sodium retention were uniformly seen with the 19-nor compounds and methyl testosterone, but not with the 19-methyl compounds.

These studies are now being prepared for publication.

Proposed Course of Project:

Analytical work is being done on a number of other studies on patients with osteoporosis. Further studies will depend on results.

Part B included Yes No

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B:

Publications:

Bartter, Frederic C., Osteoporosis, American Journal of Medicine, XXII: 797-806, No. 5, May, 1957.

Serial No. NHI-46
1. General Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title

The Effect of Bovine Growth Hormone in Man

Principal Investigator: Werner, I., M.D.

Other Investigators: Biglieri, E.G., M.D., and Bartter, F.C., M.D.
Delea, D., Diller, E., Henderson H.

Man Years
Total .97
Professional: .27
Other: .70

Patient Days: 400

General Purpose:

Bovine growth hormone has been found to be generally ineffective in man. Russell and others have found that the effect of bovine and other growth hormones in inducing nitrogen retention is very much greater in animals when fat forms a large portion of the caloric intake. The present studies were designed to test whether the same applies to man. Growth hormone has been reported to increase secretion of aldosterone and these studies were also designed to determine whether this could be confirmed. In particular, we sought to determine whether the spontaneous decrease of aldosterone secretion with continued ACTH therapy could be obliterated with simultaneous use of a growth hormone.

Major Findings:

Six "normal controls" and one obese man were studied on metabolic regimen. Design of experiment included control periods and periods with growth hormone and in some subjects periods with ACTH and with ACTH and growth hormone combined.

In studies carried out in the summer, growth hormone produced significant nitrogen retention on a diet high in fat and low in carbohydrate. The results were most marked in two "obese normal controls". In one of these subjects, growth hormone appeared to diminish the nitrogen loss induced with ACTH. In studies carried out in the winter, however, including those on the obese male, growth hormone was without effect. Growth hormone did induce

moderate elevation of aldosterone secretion with some sodium retention in the majority of subjects. One normal subject was given human growth hormone on the same dietary regimen for comparison. The relative nitrogen induced by the hormone was much greater than that reported by others with this dosage except for a single case of an obese woman.

The effect of growth hormone was tested in two normal girls who received all their day's carbohydrates in the morning and their growth hormone at night (in Cuthbertson's hands this procedure greatly enhanced the nitrogen continuing effect of growth hormone). Again bovine growth hormone appeared without effect when given on this regimen.

Proposed Course of Project:

These studies have been completed and are being written up.

Part B included Yes No

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part B:

Publications:

Werner, I. and Bartter, F.C., Protein Anabolic Effects of Bovine Growth Hormone in Man, 40th Annual Mtg. Proc. Endocrine Soc., San Francisco, 1958.

Serial No. NHI-47
1. Gen. Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Maryland

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A:

Project Title: The Role of Adrenal Cortical Steroids in the Salt Retention of the Edematous State.

Principal Investigator: F. C. Bartter, M.D.

Other Investigators: F. G. Biglieri, M.D., J. R. Gill,Jr., M.D., I. H. Mills, M.D., C. S. Doles, C. Kirby, G. Smith, A.G.T. Casper, and H. Henderson.

Man Years

Total:	2.33
Professional:	.47
Other:	1.86

Patient Days: 600

General Purpose:

To evaluate the role of the adrenal cortex in the sodium retention of edema:

1. To investigate the stimuli to aldosterone secretion.

2. To evaluate the role of aldosterone in pathological sodium retention.

3. To elucidate the mechanism of action of aldosterone on the kidney.

4. To evaluate the action of agents with a potential influence on the secretion of aldosterone.

Major Findings:

1. The effect of potassium in elevating aldosterone secretion and of potassium depletion in lowering aldosterone secretion was investigated in normal subjects. It was shown that aldosterone secretion could be elevated upon loading and decreased with potassium depletion without evidence of concomitant reciprocal changes in intravascular volume.

In studies with dogs, it was shown that caval constriction would consistently elevate aldosterone secretion whether the vagi were intact or not, but that the fall of aldosterone secretion following release of caval constriction occurred only when the vagi were intact. It was found that constriction of the common carotid arteries consistently produced elevation of aldosterone secretion unless the arteries had been previously denervated.

2. The studies of the role of aldosterone in potassium depletion have been described in another progress report. Three patients with primary aldosteronism have been studied. It appears likely that the failure of such patients to lower aldosterone secretion in response to expansion of intravascular volume may be of value in differential diagnosis.

3. In three subjects balance or clearance studies were done to compare the effect of aldosterone alone on urinary sodium potassium and hydrogen secretion with that of aldosterone and spironolactone (8109). The actions of aldosterone could be blocked with the lactone.

4. The pharmacologic agent 4885 was given to several patients with secondary aldosteronism and to one patient with primary aldosteronism. It is striking that in secondary aldosteronism, urinary aldosterone was lowered but there was no sodium diuresis; desoxycorticosterone secretion appeared.

Proposed Course of Project:

Studies are being pursued along all these fronts.

Part B included Yes No

Part B:

Publications:

1. Bartter, F.C., Biglieri, E.G., Pronove, P., and Delea, C.S. Effect of Changes in Intravascular Volume and Aldosterone Secretion in Man. Ciba Symposium on Aldosterone, 1957.

2. Bartter, F.C., The Role of Aldosterone in the Regulation of Body Fluid Volume and Composition. The Scand. J. of Clin. and Lab. Invest., 1957, 10, Suplementum 31.

3. Bartter, F.C., The Physiological Control of Aldosterone Secretion. Proc. of the Royal Soc. of Med., 51, 201-202, No. 3, March 1958.

4. Bartter, F.C., Mills, I. H., Biglieri, E.G., and Delea, C.S.. Studies on the control and Physiologic Action of Aldosterone. Recent Progress in Hormone Research, 1958, In press.

5. Mills, I.H., Casper, A.G.T., and Bartter, F.C. On the Role of the Vagus in the Control of Aldosterone Secretion. Science 128, No. 3320, p. 1140, 1958.

Serial No. NHI-48
1. General Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Maryland

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Ultrafiltration studies of steroid protein binding.

Principal Investigator: P. S. Chen, Jr., M.D.

Other Investigators: H. P. Schedl, M.D., F. C. Bartter, M. D.
G. S. Green, H. Smith, C. S. Delea, D. Berkant.

Man Years

Total:	2.03
Professional:	.86
Other:	1.17

Patient Days: 200

General Purpose:

Many steroids are bound to plasma proteins. The steroids differ in the extent to which they are bound and in the strength of the bond. At least two different plasma proteins are involved in steroid binding. Under certain physiological conditions, the degree of binding (and presumably the amount of binding protein) undergoes change. The present studies are designed to study protein binding of all steroids, to study the factors which alter protein binding, and to evaluate the proposition that only the unbound fraction is physiologically active.

Major Findings:

Large quantities of plasma were ultrafiltered by the Toribara technique at 37° C. and the extent of binding of hydrocortisone estimated chemically. Five to 10% of the plasma hydrocortisone of normal subjects was found to pass through the membrane. A procedure was developed for acquiring the same information from small samples by adding radioactive steroid to the plasma in determining the amount ultrafilterable with the use of the double labelled isotope derivative method of Peterson and Kliman. The information obtained could be greatly increased by first suppressing the secretion of hydrocortisone with active synthetic steroids to negligible levels and then adding several different amounts to a given specimen of plasma and acquiring a "titration curve".

These techniques were used to measure the effect of estrogens, of fasting, of Nilevar (17-alpha-ethyl 17-hydroxy norandrostenone), and of surgical trauma on the degree of plasma binding of hydrocortisone. Preliminary results indicate (1) that estrogen markedly increases plasma hydrocortisone almost entirely through effect of increasing bound plasma hydrocortisone and thus presumably the amount of specific hydrocortisone-binding protein, (2) fasting may produce a similar increase in specific steroid binding, (3) Nilevar probably does not have such an effect on steroid binding, (4) not enough results are available for surgical trauma as yet. The specific binding of hydrocortisone in fetal plasma was found to be very small, a finding quite in keeping with the very low level of total hydrocortisone found in fetal plasma.

Using isotope technique, preliminary studies have been done to evaluate renal clearance of hydrocortisone by comparing the clearance of ultrafilterable steroid with that of inulin. The results suggest that several times as much plasma free hydrocortisone is filtered as is excreted in the urine.

All labelled steroids obtainable from the Endocrinology Study Section and a number of others labelled by Wilzbach's technique and purified here have been examined for degree of binding to albumin, Cohn fraction 5, or to plasma. Results indicate that plasma binding of aldosterone, progesterone, and androstenedione is accounted for by the attachment of these steroids to albumin alone, whereas, a specific corticosteroid binding protein (probably an α-globulin) strongly binds hydrocortisone, cortisone, corticosterone and compound S. With these steroids, appreciable binding to albumin appears only after the specific binding a steroid is saturated.

Proposed Course of Project:

The study is being extended to clarify the role of fasting, trauma, estrogens, and other steroids on specific plasma binding. Studies include determination of hydrocortisone pool size during the change induced by these agents.

Studies with different steroids are being continued in the attempt to elucidate the relationship of steroid structure to specific binding of steroid.

Part B included Yes No

Part B:

Publications:

1. Mills, I.H., Schedl, H.P., Redd, D., and Bartter, F.C., The Determination of Non-protein Bound Plasma Hydrocortisone by Ultrafiltration. 40th Annual Mtg. Proc. Endo. Soc., San Francisco, 1958.

2. Chen, P.S.,Jr., Mills, I.H., Bartter, F.C., A Simple Ultrafiltration Method for Determining non-protein Bound Steroids (Abstract) IV International Congress of Biochemistry, Vienna, Austria (1958), p. 119.

3. Chen, P.S.,Jr., Liquid Scintillation Counting of C^{14} and H^3 in Plasma and Serum. Proc. Soc. Exp. Biol. & Med. 98, 546-547 (1958).

4. Mills, I.H., and Bartter, F.C., Plasma-Hydrocortisone levels during Cortisone Administration. Lancet, II, 1958, p. 941.

5. In addition, results were presented in some detail by Mills, I.H., Discussion of Lieberman's paper entitled "Steroid protein conjugates", Laurentian Hormone Conference, Sept. 9, 1958. This discussion is of importance in that it includes material explaining the failure of previous workers to measure ultrafilterable hydrocortisone.

Serial No. NHI-49
1. General Med. & Exp. Therap
2. Clinical Endocrinology
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

Studies on Vitamin D.

Principal Investigator: Chen, Philip S., Jr., Ph.D.

Other Investigators: None

Man Years
Total: .20
Professional: .20
Other:

Patient Days: None

Major Findings:

An effort was made to obtain tritium labelled Vitamin D_3 by the gas exposure technique for metabolic and in vitro studies. Several trials resulted in complete failure; small amounts of Vit. D_3 were destroyed, larger amounts were partially destroyed and the Vit. D_3 was devoid of incorporated tritium. The radioactivity present (from Tracerlab or New England Nuclear) in the irradiated Vit. D_3 mixture was associated with a more polar compound running faster on the reversed phase Kodicek paper chromatographic systems.

Proposed Course of Project:

An attempt will be made to identify the radioactive breakdown product. Further work will be done in trying to obtain labelled Vit. D_3.

Part B included Yes No

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

Studies on the Determination of Urinary Aldosterone

Principal Investigator: Chen, Philip S., Jr., Ph.D.

Other Investigators: None

Man Years
Total: .20
Professional: .20
Other:

Patient Days: None

Major Findings:

Chemical studies on the use of salicyloyl hydrazide have led to the following conclusions. The compound does not form useful fluorescent derivatives with the corticosteroids. Derivatives of the Δ^4-3 ketosteroids, however, do possess useful U.V. spectra. Some characteristics of the reaction of salicyloyl hydrazide with Δ^4-3 ketosteroids were studied and an application of the technique was made to urinary aldosterone.

Proposed Course of Project:

The development of the isotope derivative method for aldosterone made available a method of greater sensitivity which also corrected for chromatographic recoveries. Consequently, the use of the salicyloyl hydrazide method as a routine procedure was not adopted. It remains a useful chemical reagent for characterization.

Part B included Yes No

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part B:

Publications

1. Chen, P. S., Jr., Fluorescence of Some Salicyloyl Hydrazones, Accepted for Publication by Anal. Chem. on October 20, 1958.

2. Chen, P.S., Jr., Spectrophotometric Determination of Δ^4-3 Ketosteroids with Salicyloyl Hydrazide. Application to Determination of Urinary Aldosterone. Accepted for publication by Anal. Chem. on October 20, 1958.

Serial No. NHI-51
1. General Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

Conversion of Progesterone -4-C^{14} to Aldosterone by the Perfused Adrenal Gland

Principal Investigator: Chen, Philip S., Jr., Ph.D.

Other Investigators: None

Man Years
Total: .20
Professional: .20
Other:

Patient Days: None

Major Findings:

It was proved that aldosterone-C^{14} was produced from progesterone-4-C^{14} by the perfused calf adrenal gland. Radioactive steroid which migrated on paper chromatograms like aldosterone was mixed with authentic tritium ring labelled aldosterone (from Dr. Ralph Peterson) and non-radioactive aldosterone. The mixture was subjected to paper chromatography in five systems, including acetylation after the third system. Liquid scintillation counting showed that the aldosterone spots consisted of material with a "constant isotope ratio", proving the identity of the C^{14} labelled steroid.

Proposed Course of Project:

There was insufficient aldosterone-C^{14} to use for metabolic studies. This project has been terminated.

Part B included Yes No

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part B:

Publications:

Chen, P. S., Jr., Schedl, H. P., Rosenfeld, G., Bartter, F.C. Conversion of Progesterone -4-C^{14} to Aldosterone by Perfused Calf Adrenals, Proc. Soc. Exp. Biol. Med., 97, 683-685 (1958).

Serial No. NHI-52
1. General Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

The Colorimetric Determination of Magnesium

Principal Investigator: Chen, Philip S., Jr., Ph.D.

Other Investigators: None

Man Years
Total: .20
Professional: .20
Other:

Patient Days: None

Major Findings:

A suitable routine procedure has not been developed, but further knowledge about the mechanism of color formation was obtained. The violet color formed when ammonia is added to Eriochrome Cyanine R and $Mg.^{++}$ seems to be due to a "lake". A large temperature coefficient is explainable on the basis of the solubility of magnesium hydroxide. The "lake" nature of the color also explains (a) the inhibition due to water and (b) the non-conformity to Beers law.

Proposed Course of Project:

An effort will be made to apply the reagent to biological samples if the reaction can be made sufficiently reproducible.

Part B included Yes No

Serial No. NHI-53
1. General Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

Water Loading in Normal and Cirrhotic Subjects

Principal Investigators: Schedl, H., M.D., Ph.D., Bartter, F. C., M.D.

Other Investigators: G. Greene

Man Years
Total: .60
Professional: .43
Other: .17

Patient Days: 200

General Purpose:

To investigate a mechanism for the subnormal free water clearance of patients with cirrhosis. To determine the role of proximal tubular sodium reabsorption and antidiuretic hormone in producing this defect. To elucidate the mechanisms involved by producing a comparable syndrome in normal subjects.

Major Findings:

Using 20 ml./Kgm. oral and intravenous water loads: The diuretic response of the normal subject has been shown to decrease progressively as salt depletion is intensified. An impaired diuresis of free water comparable with that shown by a population of decompensated cirrhotics is seen in the well hydrated normal subject after intensive salt depletion.

Using 20 ml./Kgm. intravenous loading: The infusion of mixed isotonic solutions of mannitol and hexoses (dextrose and fructose) has been shown to increase free water clearance in both cirrhotics and salt depleted normals above that seen with an equal load of the hexose alone. Both groups show a comparable degree of "correction" of the impaired diuretic response to intravenous hexose when the mannitol mixture is used.

No free water is formed when isotonic (5%) mannitol is infused into the normal subject under these conditions. The decompensated cirrhotic and the salt depleted normal do show positive free water clearance under these conditions.

Using sustained intravenous loading in decompensated cirrhotics and in normal and salt depleted control subjects: Relations between free water clearance and the inulin clearance and the effects of substituting mannitol or saline during loading have been studied. Free water clearance was found to show a change generally paralleling the clearance of inulin. The mannitol and saline increased free water clearance whenever release of antidiuretic hormone could be ruled out.

The antidiuretic response of the cirrhotic to physiologic doses of exogenous pitressin has been studied as follows: After establishing the baseline diuretic response to intravenous loading, the studies have been repeated with the simultaneous infusion of pitressin at 10m U./Kgm./hr. The cirrhotic has been shown to behave qualitatively identically with the normal.

Proposed Course of Project:

These data have been presented as a paper and are now being prepared for publication.

Part B included Yes No

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part B:

Publications:

Schedl, H.P., and Bartter, F.C., An Explanation for and Experimental Correction of the Abnormal Water Retention in Cirrhosis, Abstract, 50th Annual Meeting, American Soc. for Clin. Invest., Atlantic City, New Jersey, May, 1958, p. 58.

Serial No. NHI-54
1. General Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

Action of Parathyroid Hormone

Principal Investigators: Pronove, Pacita, M.D., Bartter, Frederic C., M.D.

Other Investigators: C. S. Delea, G. Smith, E. Diller

Man Years

Total:	1.13
Professional:	.60
Other:	.53

Patient Days: 250

General Purpose:

1. To study the physiology of the parathyroid hormone.

2. To clarify the diagnosis of hyperparathyroidism.

Major Findings:

This year we have terminated this particular phase of the study, reported in December 1957, of the action of the parathyroid hormone and have arrived at the following conclusions:

1. Tm of phosphorus. In six (6) normal subjects the Tm of phosphorus was greater than 3 mg/min., whereas, in eleven (11) cases of hyperparathyroidism the Tm was less than 3 mg./min. in 7 and greater than 3 mg./min. in four (4) patients The latter are, therefore, within normal limits.

2. Calcium infusion test. This was evaluated according to the response of urinary phosphorus to the test. Seven normal control subjects showed a drop in urinary phosphorus with a minimum of 20% fall while their serum phosphorus rose. Of ten cases of confirmed hyperparathyroidism, 4 showed a drop from 2.6% to 27.9% while 6 showed a rise above their normal values with very small increase in serum phosphorus. There is, therefore, a slight overlap in results between normal subjects and those with hyperparathyroidism.

3. Amphojel test. This test has proven to be of good diagnostic value in the diagnosis of hyperparathyroidism so far. Twelve patients with confirmed parathyroid tumors (10) or hyperplasia (2) had "positive" results. This means

that their excretion of calcium in the urine was greater than their intake of 200 mg./day. The minimum rise of 250 mg./day occurred in both cases of hyperplasia. In patients with tumors, serum calcium rose to 11 mg./% with amphojel, but in those with hyperplasia it rose only to 10.3 mg./% and 10.5 mg./%, respectively. The serum phosphorus fell below 3 mg./% in 11 of the 12 patients (exception 3.2 mg/%).

Among the normal controls, 24-hour urinary calcium excretion was never greater than their intake of 200 mg./d., and in none did the serum calcium rise higher than 10.9 mg./%.

Many cases of kidney stone formers were subjected to the above tests and all of them responded in a manner similar to the normal controls.

Proposed Course of Project:

This particular phase of study of parathyroid activity has been terminated and all the data are being accumulated for publication in the near future.

Part B included Yes No

Serial No. NHI-55
1. General Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

Aldosterone Metabolism in Autonomic Nervous System Dysfunction with Postural Hypotension.

Principal Investigator: Bartter, Frederic C., M.D.

Other Investigators: E. G. Biglieri, M.D., D.S. Gann, M.D., and J. R. Gill, Jr., M.D. Technical: C. Delea, D. Berkant, E. Diller, and H. Henderson.

Man Years

Total:	1.47
Professional:	.90
Other:	.57

Patient Days: 200

General Purpose:

Stimuli such as potassium loading or deprivation, ACTH, and contraction and expansion of ECF or intravascular volume consistently affect aldosterone secretion in normal subjects. The present study is an attempt to determine whether patients with postural hypotension and autonomic insufficiency have similar mechanisms for control of aldosterone secretion.

Major Findings:

In 5 patients with autonomic insufficiency and postural hypotension, localization of the lesion was attempted by measurement of response to Valsalva maneuver and to mental arithmetic, response of skin temperatures to peripheral nerve block, response of pupils and blood vessels to small doses of epinephrine, norepinephrine, and other sympathicomimetic drugs. With metabolic balance techniques, 24-hour urinary excretion of aldosterone was studied during potassium loading, during sodium depletion, with expansion of intravascular volume with albumin, and with ACTH. All subjects tolerated potassium poorly, showing abnormal rises in serum potassium and small rises in aldosterone secretion. Response to salt deprivation varied markedly, as did the excretion of aldosterone; it appears likely that the location of the lesion determines the degree of impairment of the response.

Proposed Course of Project:

These studies are being continued until it is possible to determine the exact nature of the defect in patients with postural hypotension.

Part B included Yes No

Serial No. NHI-56
1. General Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

On the Nature of Potassium Losing Renal Disease

Principal Investigators: Bartter, Frederic C., M.D., and Gill, John R., M.D.

Other Investigators: Delea, C. S., Diller, E., Middleton, H., Turner, R., Berkant, D., and Kirby, C.

Man Years:

Total:	1.60
Professional:	.27
Other:	1.33

Patient Days: 250

General Purpose:

Hypokalemia associated with urinary potassium loss appears in a number of syndromes. The present studies were instituted to determine what features these syndromes have in common, and in particular the role of aldosterone in the renal potassium loss. Although for the purposes of the present study patients with primary aldosteronism have been excluded, the studies point out the difficulty of establishing clearly the difference between primary and secondary aldosteronism.

Major Findings:

Two groups of patients have been studied: those with associated alkalosis (2), and those with associated renal tubular acidosis (3). Studies have included balance studies with special reference to sodium, potassium and hydrogen ions and renal clearance measurements.

In both groups of patients, inability to conserve sodium and elevated urinary aldosterone have been demonstrated. It was clear nonetheless that excessive potassium loss could occur when sodium was not being lost in the urine. Conversely, severe salt loss was observed together with the ability to maintain normal potassium balance. When the extracellular and intravascular fluid volumes were expanded in these

subjects, there was no clear evidence that aldosterone secretion was diminished even though serum potassium was restored to normal.

Proposed Course of Project:

It is necessary that we determine whether the aldosteronism is autonomous or secondary to factors in these subjects. The effect of agents known to alter aldosterone secretion, including metabolic blocking agents, is therefore being studied.

Part B included Yes No

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

Calcium Metabolism in Sarcoidosis

Principal Investigators: Gill, John R., M.D., and Bartter, Frederic C., M.D.

Other Investigators: C. Delea and Staff

Cooperating Units: These studies have been done in conjunction with Dr. Norman Bell, (NIAID, LCI).

Man Years
Total: .93
Professional: .23
Other: .70

Patient Days: 150

General Purpose:

Patients with sarcoidosis frequently have hypercalciuria even on a low calcium intake. It has been suggested, but not proved that (1) hypercalciuria is secondary to increased calcium absorption, (2) the increased absorption is a result of hypersensitivity to vitamin D, (3) steroids with carbohydrate activity will decrease the hypercalcuria, and (4) this action of the steroids operates by blocking sensitivity to vitamin D.

Major Findings:

Metabolic balance studies have been carried out in three patients with sarcoidosis. Determinations included calcium balance, phosphorus balance, nitrogen balance and periodic determinations of serum, calcium, phosphorus, and vitamin D. The design of experiment includes periods of added calcium plus vitamin D, added calcium plus prednisone and all three treatments. A large number of patients have been surveyed to determine their suitability for this program. Determinations have included Tm of phosphorus, response of the serum and urine calcium and phosphorus to amphojel on a low phosphate diet, and response to calcium infusion.

Results of the balance studies are still in process of analysis. Preliminary data indicate that (1) patients with sarcoidosis differ markedly in degree of calcium absorption, (2) when these patients do show hypercalciuria with low fecal calcium they may exhibit normal blood levels of vitamin D, (3) In patients with a low fecal calcium and hypercalciuria, prednisone may effectively diminish the hypercalciuria.

Proposed Course of Project:

Collection of sufficient data, bearing on the points outlined above, to produce a definitive conclusion.

Part B included Yes No

Serial No. NHI-58
1. General Med. & Exp. Therap.
2. Clinical Endocrinology
3. Bethesda, Md.

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title:

Study of Movement of Proteins and Lipids Through Arterial Walls and Similar Tissues

Principal Investigator: Duncan, L. E., Jr., M.D.

Other Investigators: Buck, K., and Lynch, A. (Technical)

Man Years:

Total:	2.50
Professional:	1.00
Other:	1.50

Patient Days: None

Objectives:

An understanding of the processes involved in the movement of proteins and lipids into and out of arterial walls and similar tissues.

Major Findings:

The work has been carried out by studies in rabbits and dogs. Since no method existed for determining the transfer rates of substances into and out of tissues when their transfer rates are slow, our early studies were devoted to working out such methods. In these early studies the movement of labeled albumin through rabbit aorta and tissues which were in some way morphologically similar to aorta was studied.

Following this the movement of labeled albumin into and out of the aorta of the dog was studied. The larger size of this artery made a more detailed analysis possible. The aorta was divided into a number of areas and each area was split into inner, middle, and outer layers. The data obtained support the concept that proteins move from blood in the aortic lumen across the intimal endothelium into the inner layer of the aorta. A striking gradient of inflow rates for albumin was found. In the proximal aorta near its origin from the heart, albumin moves into the aortic wall very rapidly. This inflow rate decreases progressively down the length of the aorta. The outflow rates do not exhibit any such gradient but are the same along the length of the aorta. Thus, the concentrations of albumin in the proximal aortic wall are higher than they are in the distal aortic wall.

Knowledge of the factor causing this gradient would be a matter of some interest, since it appears that this factor is a major determinant of the rate of movement of proteins into arterial walls. The gradient of inflow rates is not caused by differences in lateral blood pressure since this is the same along the length of the aorta. However, since the diameter of the aorta decreases progressively along its length, the circumferential tension to which the aortic wall is subjected would tend to decrease proportionately as described by Laplace's law. Current theory holds that proteins pass through endothelium by moving through pores in the intercellular cement which joins the endothelial cells rather than by passing through the bodies of the cells themselves. It appears possible that the greater circumferential tension in the proximal aorta separates the endothelial cells more widely there than elsewhere and thus permits proteins to flow through more rapidly.

This work has been extended to the study of the movement of radio-cholesterol through the canine aorta. These data present certain difficulties in interpretation that the data for radioalbumin did not. However, it appears clear that radio-cholesterol shows the same type of gradient of incorporation rates along the length of the aorta that radioalbumin does. It also appears that the rate of movement of radio-cholesterol out of the distal aorta is much slower than the rate of movement out of the proximal aorta, so that the concentrations of radio-cholesterol in the distal aorta eventually exceed those in the proximal aorta.

Significance to heart research: A widely held theory is that atherosclerosis develops by the movement of lipids from serum into arterial walls either as part of lipoproteins or by some other mechanism. Quantitative study of the movement of proteins and lipids into and out of arterial walls should increase our knowledge of the variables and mechanisms involved in the process of atherosclerosis.

Proposed Course of Project:

Currently, we are beginning to apply our techniques to the study of the movement of cholesterol into the arteries of animals with elevated serum cholesterol levels. We hope to be able to study this process in both rabbits and dogs. The practicality of iodinating lipoproteins and following their movement into arterial walls is also being investigated.

Part B included Yes No

PHS -- NIH
Individual Project Report
Calendar Year 1958

Part B:

Publications

1. Leroy E. Duncan, Jr., Jerome Cornfield, and Katherin Buck, Circulation of Iodinated Albumin Through Aortic and Other Connective Tissues of the Rabbit. Circulation Research 6: 244, 1958.

2. Leroy E. Duncan, Jr., Jerome Cornfield, Katherin Buck, Circulation of Labeled Albumin Through the Aortic Wall of the Dog. Accepted for publication by Circulation Research.

Form No. ORP-2
Oct. 1957

PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
Laboratory of Gerontology

Estimated Obligations for FY 1959

Total: $632,344

Direct: $480,000

Reimbursements: $152,344

GERONTOLOGY BRANCH

Project Report

September 1, 1958 - December 31, 1958

1. a. Title: Studies on the mechanism of oxidative phosphorylation.

   b. Principal Investigator: D. Rao Sanadi (1/2 time)

      Technical Assistance: Samuel Crowder

   c. Progress During Past Four Months: This is a new project initiated in September 1958.

   d. Direction of Current Research: We had previously found that cadmium ions at extremely low levels would uncouple phosphorylation from oxidation. The effect was reversed by dithiols but not by monothiols. These results suggested that a dithiol might be involved in the coupled phosphorylation.

      Our immediate plans call for testing in mitochondria the effect of Cd^{++} on various reactions that are apparently closely related to phosphorylation, e.g., ATPase activity under various conditions and phosphate-ATP exchange. We also propose to study the effect of Cd^{++} in sub-mitochondrial fragments which have retained their capacity to carry out oxidative phosphorylation.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by D. Rao Sanadi
October 27, 1958

d. Direction of Current Measure: We had previ
mum tons at extremely low levels would
from oxidation. The effect was reverse
results indicated
tion is then complet

GERONTOLOGY BRANCH

Project Report

January 1958 - December 1958

1. a. Title: Choice reaction time as a function of aging.

Goal: To investigate age decrement in reaction time with reference to stimulus complexity. In general terms, the hypothesis being tested is that age decrement increases as a function of increasing stimulus information.

Method: Originally the procedure was to have subjects learn to name each of eight symmetrically placed lights with one of eight non-sense syllables. After achieving a certain learning criterion, the subject was to react to one light in each of a series of subsets of the eight lights. The subsets consisted of combinations of 1, 2, 3, 4 or 5 lights. A series of 24 of each subset was presented. Reaction time was measured by a 1/100 second timer which started with the presentation of one light and stopped when the subject uttered a non-sense syllable. The amount of information in bits was calculated for each subset of lights by finding $\log_2 x$, where $x = 1, 2, 3, 4, 5$. The reaction times are averaged over the 24 presentations of each subset for each subject. Each subject produced, then, a relationship between information in bits and reaction time. These relations, by inference from another study are supposedly linear, but this assumption needs to be tested. The prediction is that the older subjects produce linear regressions but with a steeper slope than the younger; in other words, the older subjects take relatively longer to decide which light has come on when the possible number of alternatives increases.

On the basis of experience indicated under "Progress", certain changes in method are now being instituted. The number of lights to be learned is being changed from eight to four. The subset of lights will now be presented with the lights on rather than off. The stimulus will now consist in having one light go off. The reasons for these changes are indicated below.

b. Principal Investigators: George J. Suci (1/4 time)
Walter W. Surwillo (1/5 time)
Melvin D. Davidoff (1/4 time)

Technical Assistance: None

c. Progress During Past Twelve Months: The apparatus for this study was constructed by the investigators and a number of young and old subjects were run. The results to date are ambiguous in regard to linearity and age differences are not reliable with the N involved. The original procedure involved such a strenuous learning task, that the learning procedure plus the experimental runs required sessions of considerable duration on each of two successive days. Getting subjects to volunteer and, once willing, to sustain a high level of motivation was a real problem. We have therefore decided to change the procedure as indicated above. The new procedure should also result in better experimental control of the subject's keeping a particular subset in mind as the source of the oncoming stimulus.

d. Direction of Current Research: The revision of the apparatus having been completed, we are about to run pilot subjects through on the revised procedure in order to obtain running time estimates and solidify procedure.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Melvin D. Davidoff
October 30, 1958

Serial No.: NHI-61

GERONTOLOGY BRANCH

Project Report

January 1958 - December 1958

1. a. Title: Memory functions as related to age.

Goal: To study memory for different types of material and sense modalities as a function of aging. Three hypotheses have so far been under investigation:

i. Aged subjects' short span memories are more susceptible to interference than younger subjects.

ii. Measurement of vocabulary retention with recall techniques show greater deterioration with age than measurement with recognition techniques. The project involving Hypothesis ii is being temporarily dropped for want of appropriate older subjects. Data on the younger subjects have been collected. Hypothesis i derives from the work of Welford and others, and is motivated by the ambiguity of experiments with the aged using the negative transfer paradigm.

iii. The retention of verbal sequences is a function of sequence length, redundancy in the sequence, and age.

Method: Hypothesis i: The amount of interference as measured by anchoring effect on judgments of sizes of squares is to be observed for subjects of different ages. A tenable hypothesis would predict greater anchoring effect for older subjects. Subjects are to learn to discriminate between five different sized squares. Then five larger squares are introduced for discrimination. The first five squares are then judged again. Only subjects who can learn to discriminate among the original set of squares within a given number of trials are retained. A control group of both young and old does not receive the interpolated set of squares, but instead are given an interpolated pause equivalent to the time taken for judgment of the interpolated set in the experimental group. One can then observe whether the interpolated set is actually interfering with the memory of the first set, or whether such deterioration would have occurred over a similar period of time anyway. The effect is measured by the number of trials necessary to return to the level of discrimination established in the first set of judgments.

Date of commencement: Summer of 1957.

Hypothesis iii: Word lists, ten, fifteen, twenty and twenty-five words in length, and of varying redundancy (such that each word occurs independently of the last in the list, two words are dependent but independent of the next pair, and so on through seven word dependency) have been constructed. Each list is read to the subject at the rate of one word per second.

The subject is asked to repeat as many words as he can remember. Scoring is in terms of some function of the number of words recalled correctly. Analysis is in terms of the interactions and primary effects of the variables of age length and redundancy. Since these same data will be used in the test of another hypothesis (see Verbal Performance study), each subject is tested individually and repetitions are recorded on magnetic tape. Appropriate controls re possible effect of order of presentation of the word lists are incorporated in the study.

b. Principal Investigators: George J. Suci (1/4 time)
Melvin D. Davidoff (1/4 time)

Technical Assistance: Jesse Yaffa (1/3 time since September)

c. Progress During Past Twelve Months:
i. Data on 62 experimental and 34 control subjects have been obtained. This has concluded our data collection. The data have been analyzed with strong confirmation of the hypothesis.
iii. Twelve subjects in each of three age groups have been run. Data collection is continuing.

d. Direction of Current Research:
i. A paper describing this study is being written. After this paper is finished further work in this area will be instituted as time permits.
iii. Collection of data is continuing.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals and the Veterans Administration Hospital at Fort Howard, Maryland.

4. Publications and Awards: None

Prepared by Melvin D. Davidoff
October 30, 1958

Serial No.: NHI-62

GERONTOLOGY BRANCH

Project Report

January 1958 - December 1958

1. a. Title: Age changes in renal physiology.

Objectives: The objectives of this project are to describe and elucidate the mechanisms of age changes in renal function.

Methods Employed: Standard clearance methods for the estimation of G.F.R., R.P.F., and T_m are used. Determinations of renal concentrating ability are being performed using the Fishberg Technique with determination of urine osmolarity by freezing point depression.

The Wallenius Method of measuring glomerular permeability employing dextrans of various molecular weights will be used to determine possible age changes in glomerular permeability.

Hemoglobin clearance is being reinvestigated in the light of newer knowledge related to the binding of hemoglobin in a hemoglobin-haptoglobin complex.

b. Principal Investigators: D. A. Cursier (Full time)
M. J. Yiengst (3/4 time)
J. Lowenstein (1/4 time)
D. A. Olewine (1/4 time) (E. O. D. Aug. 4, 1958)

Other Investigator: N. W. Shock

Technical Assistance: Ramona Dorcas (Full time)
Theresa Caryk (3/4 time)
Llewellyn Perkins (Full time)
(E. O. D. Apr. 28, 1958)

c. Progress During Past Twelve Months: Repeat tests of renal function by standard clearance techniques are being continued in connection with the longitudinal program. The number of tests and the intervals between repeat tests are still too small to draw any conclusions. Renal functions are being performed on all male Baltimore City Hospitals infirmary admissions who are likely to be permanent residents. It is planned that they will have repeat tests at regular intervals (Cursier).

Work on the dextran technique is continuing (Oursler and Yiengst).

A number of dextran fractions with a restricted band of molecular weights have been prepared by reprecipitation techniques for calibration purposes (Oursler and Yiengst).

Nineteen male infirmary patients (ages 48-75) have been followed on a Fishberg type dehydration study in which urine osmolarity was determined on samples collected at 3 hour intervals after a control sample at 6:00 p. m. and continuing until 6:00 a. m. Fifty-five observations of urine osmolarity were made. In almost half of the cases the control sample (6:00 p. m.) or 3 hour sample (9:00 p. m.) urine osmolarity was equal to or greater than the 6:00 a. m. sample. Urine/plasma ratios were calculated for this series of patients. (P = 285, range 278-291). Using the highest u/P found on two replicate tests for each patient we noted a u/P of from 1.59 to 4.28 with an average of 3.08. If only the highest u/P is noted for each patient, the average becomes 3.45. The inference is that some of these people may be chronically dehydrated. Further experiments are underway to clarify the situation. Outpatients who are being tested in several other studies are also being investigated and will be compared to our infirmary population (Oursler and Yiengst).

Work is now in progress to perfect a technique for the electrophoretic separation of free and bound hemoglobin (Lowenstein).

The urine osmolarity was compared in two groups of Wistar strain rats aged 19 and 26 months which were maintained on an unrestricted food and water intake. Average values for 14 animals in each age group were compared based on a mean of 3 observations from randomly collected morning urines. Urine osmolarity in the older rats was significantly lower (1454 milliosmoles per liter, i. e., ± 101) than in the 19 month old animals (1905 ± 80) (P = .002).

The urinary protein concentration was compared with the urine osmolarity on some of the animals in the above study. The data indicate that an inverse relationship may exist between renal concentrating ability and proteinuria--that is, as the animal's ability to secrete a concentrated urine diminishes there is an increase in urine protein (Yiengst and Olewine).

d. Direction of Current Research: Renal clearance studies are being continued. Clearances are being done on satisfactory

male infirmary admissions and will be repeated at regular intervals. Another series of patients are being examined by the urine concentration technique. Dextran excretion as an index of glomerular permeability will be followed as soon as the methodology is complete (Oursler and Yiengst). Hemoglobin will be used in the study of glomerular permeability as well as in the reevaluation of hemoglobin clearances (Lowenstein).

Rats of various ages will be followed under stress conditions such as dehydration, water-loading, etc., to measure their concentrating ability. An attempt will be made to adapt renal clearance techniques, as used in the human, to the rat (Yiengst and Olewine).

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards:

Shock, N. W.: The role of the kidney in electrolyte and water regulation in the aged. In: G. E. W. Wolstenholme and M. O'Connor (Editors), Ciba Foundation colloquia on ageing. Vol. 4. Water and electrolyte metabolism in relation to age and sex. J. & A. Churchill Ltd., London, 1958, pp. 229-249.

Prepared by David A. Oursler
November 5, 1958

GERONTOLOGY BRANCH

Project Report

January 1958 - December 1958

1. a. Title: Age changes in human performance.

This project is designed to study the effects of aging on (a) the physiologic responses to exercise, (b) the rate of recovery of physiologic equilibrium after exercise, (c) muscular efficiency and (d) work output and fatigue. In addition, the factors responsible for limitations in performance observed in older people will be evaluated.

Measured amounts of physical work will be obtained in subjects of varying ages by means of a calibrated arm ergometer and quantitative mechanical analysis of limb movement. A treadmill will be used to induce higher levels of work. Measurements of oxygen uptake, CO_2 elimination, pulmonary ventilation volume, heart rate, blood pressure, and cardiac output (by the dye method) will be taken before, during and after standardized amounts of exercise. Each experiment involves analysis of 3-8 samples of expired air for standardization of automatic gas analyzers. Other studies will include measurements of speed of nerve conduction, reflex delay time, and muscle action potentials. These phenomena will be recorded on a six channel oscillograph or dual beam oscilloscope as the experiment demands.

This is a continuing program. One of the most important aspects of the program will be to measure physiological responses to exercise and evaluate physical performance in the same subjects at intervals of three to seven years.

b. Principal Investigators: Arthur H. Norris (1/4 time)
Joseph A. Falzone, Jr. (1/4 time)

Other Investigators: Nathan W. Shock
Norman W. LeVora
Felix Hügin

Technical Assistance: John B. Melvin (2/3 time)
Eleanor E. Howard
Edna Phillips (2/3 time)
Mae F. Moody (2/3 time)
Lorraine D. Ward

c. Progress During Past Twelve Months: The latency of superficial reflexes (plantar flexor and superficial abdominal) were measured in 14 young individuals (average age = 32 years) and 15 elderly subjects (average age = 75 years). The average latency of the plantar flexor reflex was 203 milliseconds in the young subjects and 279 milliseconds in the old subjects, while abdominal reflex latencies were 50 milliseconds for the young and 160 milliseconds for the elderly subjects. The t-test showed the differences to be significant ($p = <0.05$ and $p = <0.001$ respectively). In elderly persons the lowered excitability of these reflexes, as determined by increased latency, is felt to be due to deficiency of central excitatory processes which is reflected in effector systems governing reflex activity, rather than those related primarily to simple voluntary motor performance.

Studies of mechanics of limb movement have emphasized the relation between amplitude of swing and mechanical efficiency for a series of rapid back and forth movements of the arm around the shoulder as an axis. Forty subjects were tested at 3 or 4 different amplitudes of swing. Twenty subjects less than 40 years of age showed an increase in average mechanical efficiency with an increase in average displacement from 8 per cent efficiency at 0.4 radians displacement to 15 per cent efficiency at 1.8 radians. Twenty subjects 40 years of age and older did not follow this pattern. They increased from 5 per cent efficiency at 0.4 radians displacement to 9 per cent efficiency at 0.8 radians displacement but showed no further increase in efficiency as displacement increased to 1.8 radians. Measurement of both velocity and acceleration of the individual swings permitted the calculation of the "driven displacement" which is the portion of the displacement during which the muscles are exerting force on the limb as opposed to the portion of the displacement during which the arm is swinging free. For both old and young subjects, "driven displacement" occupies 90 per cent of the total displacement at 0.4 radians and only 50 per cent of the total displacement at 1.8 radians. The greater mechanical efficiency at high displacements is interpreted as the result of a greater proportion of "free swing" at these displacements. The differences in efficiency between the old and young subjects at the highest displacements (1.25 and 1.80 radians) are interpreted as the result of more simultaneous recruitment of greater numbers of muscle fibres in the muscles of the young subjects. This is associated with the observed greater accelerations and higher swinging rates in the young subjects.

d. Direction of Current Research: Measurements of plantar and abdominal reflex latencies will be extended to subjects of a

higher socio-economic level and compared with measurements of speed of nerve conduction made in the same individuals. The studies of limb mechanics will be extended to greater numbers of subjects in the same select groups of old and young subjects with emphasis on monitoring the rate of work performance and the timing of recorded muscle action potentials.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

Dr. Robert W. Ramsey, Medical College of Virginia, Richmond, Virginia

Dr. John W. Magladery, The Johns Hopkins University, School of Medicine, Baltimore, Maryland

Dr. Robert D. Teasdall, The Johns Hopkins University, School of Medicine, Baltimore, Maryland

4. Publications and Awards:

1. Magladery, J. W., R. D. Teasdall, and A. H. Norris: Effect of aging on plantar flexor and superficial abdominal reflexes in man--a clinical and electromyographic study. J. Geront., 13: (3), 282-288, July 1958.

Prepared by Arthur H. Norris
October 31, 1958

Serial No.: NHI-64

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Adaptive enzymes and age.

   b. Principal Investigator: Robert I. Gregerman (1/1/58 - 6/30/58) (1/3 time)

   Technical Assistance: James Tucker (1/4 time)

   c. Progress During Past Twelve Months: Two of the adaptive enzymes of liver, tryptophan peroxidase and tyrosine transaminase, have been determined in mature (12-13 months old) and senescent (24-26 months old) rats. Basal levels of the two enzymes, the response of tryptophan peroxidase to substrate administration, and of both tryptophan peroxidase and tyrosine transaminase to adrenal cortical hormone (hydrocortisone), has been studied. In both groups of animals, the mean enzyme activities were the same in all instances. No relationship of the two enzymes to each other was observed.

   The data fail to indicate that senescence is accompanied by a biochemically demonstrable impairment of the mechanisms involved in adaptive enzyme formation and rapid protein synthesis.

   d. Direction of Current Research: This specific project is completed.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by N. W. Shock
October 31, 1958

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Age changes in cellular and tissue biochemistry.

b. Principal Investigators: Charles H. Barrows
Donald Olewine

Other Investigators: W. W. Shock
J. A. Falzone, Jr.

Technical Assistance: Lois Roeder
Frances Beran
Lawrence Valentine

c. Progress During the Past Twelve Months:

Objectives: The general purpose of this program is to examine various tissues of rats for changes associated with senescence in tissue and cellular metabolism. Specific problems investigated during this period have been:

(1) Oxidative phosphorylation in young growing rats as compared with mature and senescent animals.

(2) The effect of age on the ability of rats to synthesize various enzymes by the depletion-repletion method.

Methods:

(1) The rate of oxidative phosphorylation using succinate as the substrate has been measured in mitochondria isolated from liver by the method of Schneider and Hogeboom.

(2) Three groups of young and old rats were either (a) sacrificed immediately, (b) fed a protein free diet for 28 days or (c) fed the stock diet of our laboratory for three days following the period of protein depletion. Various enzymic activities as well as RNA, DNA and protein nitrogen of liver and kidneys of these animals were determined by accepted standard procedures.

(3) The various tissue components described above (2) were also determined in the tissues of 6 month old rats fed a protein free diet for either 0, 2, 4, 7 or 14 days.

Major Findings:

(1) A marked agewise decrement in the rate of oxidative phosphorylation occurs during growth rather than senescence. However, future studies are indicated since the rates of oxidation (oxygen uptake) and phosphorus esterified per unit of mitochondrial nitrogen were found to decrease with decreasing amounts of mitochondrial nitrogen employed in the test system (1.0 mg. and 1.8 mg. of mitochondrial nitrogen were used). Although the decrease in the oxidation per mg. of mitochondrial nitrogen, attributable to the different amounts of mitochondria used, did not appear to be dependent upon the age of the animals, the decrease in phosphorus esterification did (4%, 9%, 16% in 4 month, 12 month and 24 month old rats respectively). Future experiments will attempt to explain these observations.

(2) Experiments were carried out to test the two hypotheses: (a) an impaired protein synthesis exists in old rats and (b) there are groups of enzymes which differ in their lability during impaired protein synthesis and it is proposed that the most labile are affected by aging.

(a) No marked age differences were observed in total body weights, kidney weights or the enzymic activities of kidneys in the control, depleted or repleted state. In liver agewise decreases were only observed in the concentrations of D amino acid oxidase (33%) of both sexes and cholinesterase (33%) of female rats. Following protein depletion the concentrations of these enzymes were found to be markedly reduced and the same level of the individual enzymes were obtained regardless of age. Thus the young animals suffered a greater loss than the old. Following repletion these enzymic activities returned to the control levels (90-112%) in the old rats but not in the younger animals (64-78%). However, the absolute amount of enzyme restored was not markedly different in the two age groups. These data support the hypothesis that the concentration of some but not all enzymes of liver may decrease with age. These results further suggest that these age differences may not be due to an impaired ability of old rats to synthesize these enzymes.

(b) During short term protein depletion marked differences in the losses of the various enzymes occurred. However, these data do not offer strong evidence that the agewise differences observed in other experiments are a result of an impaired tissue protein synthesis when they are compared to the changes observed in slightly to moderately protein depleted rats. For example, although no age differences in the succinoxidase and protein nitrogen of liver tissue have ever been observed, marked decreases

(a)

in these tissue components were observed in rats depleted for only two days. An increase in the concentration of RNA of liver does not appear to occur in protein depleted rats but has been consistently found in old animals. Age differences in the concentration of liver cathepsin have been difficult to establish due to large individual variations in old rats, however, the concentration of this enzyme is increased 2-3 fold in old animals which have lost body weight. No increase in cathepsin of liver was observed in protein depleted rats.

(3) The success of animal experimentation is dependent upon not only adequate animal colony control regarding such factors as room temperature, food, water, pest, etc. but also upon the history of the individual animals used for experiments. Thus, the animal care of our colony has been improved to include more frequent weighings and physical examinations under a standardized system for each rat. Presently, special emphasis are being placed upon the senescent animals in order to more precisely define the nature of the body weight loss which precedes the death of the animals. In addition, standardized autopsy procedures and the use of standardized data forms have been initiated.

d. Direction of Current Research: Future experiments will include:

(1) Further studies on the oxidative phosphorylation of mitochondria isolated from livers of rats of different ages.

(2) A final investigation on agewise changes in protein synthesis by the depleted-repletion method.

(3) Studies of changes in the concentrations of enzymes in animals depleted by food restrictions.

(4) The physiological basis of terminal loss of body weight observed in senescent rats.

(5) Enzymic activity of tissues during regeneration (liver) and hypertrophy (kidney) of organs in animals of different ages.

2. Patient Days - None

3. Collaborators - Baltimore City Hospitals

4. Publications and Awards:

1. Barrows, C. H., N. W. Shock, and B. F. Chow: Age differences in cholinesterase activity of serum and liver. J. Geront., 13: 20-23, 1958.

2. Barrows, C. H., M. J. Yiengst, and N. W. Shock: Senescence and the metabolism of various tissues of rats. J. Geront., 13: 351-355, 1958.

3. Barrows, C. H., and B. F. Chow: Dietary Proteins and Synthesis of Tissue Proteins. Chap., in: A. A. Albanese (Editor), Proteins and Amino Acid Requirements, Academic Press (in press).

Prepared by Charles H. Barrows
October 31, 1958

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Studies on the occurrence, isolation and properties of lipofuscin "age" pigments from human cardiac muscle.

b. Principal Investigators: Bernard L. Strehler (30% of time)
Albert S. Mildvan (30% of time)

Other Investigator: Donald Mark, Baltimore City Hospitals

Technical Assistance: Malcolm Gee (40% of time)

c. Progress During Past Twelve Months: A method has been developed for the quantitative estimation of the lipofuscin pigment of human myocardium. The method consists of a "random shot" method of measuring relative areas (or volumes) of microscopic sections by measuring the frequency with which the points of intersection of lines on an ocular grid fall on the microscopic object in question.

Using this technique, the % volume occupied by heart age pigment has been measured for a series of hearts of various ages. It has been found (on the basis of the samples thus far investigated) that: (1) There is a linear increase in the % volume occupied by lipofuscin pigment from 0% at around age 10 to about 4% at age 100. (2) The % of myocardium occupied by muscle fibers is about 60-70% in the 0-10 year age group and drops to a nearly constant 40% in the 40+ age group. Thus, the % pigment in the myocardium is as much as 10% by volume. This inconsiderable amount is perhaps sufficient to cause some decrement in function and reserve capacity independent of other age dependent changes or pathologies. (3) The total amount of pigment per heart does not appear to be affected appreciably by heart size and moreover does not appear to be correlated with any other clearly recognized pathology. Thus, it may be considered as a pure age effect.

d. Direction of Current Research: We have isolated (in a state of high fluorescence purity) several samples of this compound. A technique for the estimation of the pigment in suspension is being developed. The IR spectrum as well as the fluorescent or phosphorescent spectrum is being measured. Elementary and group analysis will be undertaken when sufficient standards of purity have been attained.

GEOMORPHOLOGY BRANCH

Progress Report

January 1, 1958 - December 31, 1958

2. Patient Days: None

3. Collaborators: Dr. Donald Mark, Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Bernard L. Strehler
October 31, 1958

Serial No.: NHI-67

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Cardiovascular hemodynamics. III. The peripheral circulation in man.

   b. Principal Investigator: Milton Landowne

   c. Project terminated.

Prepared by N. W. Shock
October 31, 1958

GERONTOLOGY BRANCH

Project Report

January 1958 - December 1958

1. a. **Title:** Studies on mathematical-physical expressions of population mortality.

   b. **Principal Investigators:** B. L. Strehler (30%)
   A. S. Mildvan (30%)

   c. **Progress During Past Twelve Months:** The general theory of mortality described in last year's project report has been greatly expanded, developed and analysed. The present form of the equation describing the Gompertz relationship ($R = R_0 e^{\alpha t}$) is:

$$R_M = K\sqrt{\frac{\Delta H_0}{RT}(1 - \beta t - f(T)t}\; e^{-\frac{\Delta H_0}{RT}}\; e^{+\frac{\Delta H_0}{RT}[\beta + f(T)]t}$$

   where: ΔH_0 = the extrapolated resistance to death at some time $t = 0$

   T = a measure of the poorness of an environment

   t = time

   R_M = mortality rate

   R = conversion constant relating ΔH_0 and T

   β = fractional loss of ΔH_0/year due to inherent aging

   f(T) = fractional loss of ΔH_0/year due to T

$$\text{Since } R_0 = K\sqrt{\frac{\Delta H_0}{RT}}\; e^{-\frac{\Delta H}{RT}} \quad \text{and} \quad \frac{\Delta H}{RT} = \frac{\alpha}{\beta + f(T)}$$

   where R_0 and α are measured quantities it is possible to estimate the maximum rate of inherent aging (β), the

Project Report

1966 - December

minimum rate of environmentally induced "aging" $[f(T)]$ and their sum $(\beta + f(T)$. This value $\beta + f(T)$ is very close to the range of values for the regression coefficients of human functional capacities measured by Shock et al., but is derived exclusively from population statistics.

The theory also permits an evaluation of the amount of reserve functional capacity at any age and predicts an approximate inverse relationship between log of intercept (R_0) and Gompertz slope (α) thus:

$$\ln\left[\frac{R_0}{\gamma\alpha}\left(\frac{\pi}{2}\right)\right] + \ln K \cong -\frac{\alpha}{\beta + f(T)} = -\alpha\left(\frac{1}{\beta + f(T)}\right)$$

Making appropriate substitutions for R_0 and α we can estimate $\beta + f(T)$. Its calculated value for poor environments is approximately 1.25%/year and for good environments is approximately 0.7%/year. Thus normal senescence must be equal to or less than 0.7%/year which would predict a maximum life span under the best of conditions of somewhat less than 140 years.

d. Direction of Current Research: Further analysis of the consequences of this theory and a critique and comparison of other theories are presently under way.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Bernard L. Strehler
November 1, 1958

Serial No.: NHI-69

GERONTOLOGY BRANCH

Project Report

January 1958 - December 1958

1. a. Title: Longitudinal studies of human physiology and biochemistry. This project is designed to (1) facilitate repeated measurements of various physiological, biochemical and psychological variables in the same individuals as they grow older, (2) coordinate the utilization of the results of these measurements in establishing indices of aging and (3) compare the effects of socio-economic status on these results and the indices which may be derived therefrom. This is a continuing program.

b. Principal Investigators: Nathan W. Shock (Director)
Arthur H. Norris (Coordinator) (1/4 time)
Joseph A. Falzone, Jr. (Clinical Director)

Other Investigators: Melvin D. Davidoff
George J. Suci
Walter W. Surwillo
David A. Oursler
Jerome Lowenstein
Theodore H. Lundy
Albert Mildvan
Norman W. LeVora

Technical Assistance: Dora Goldblatt
Edna Phillips (1/3 time)

d. Progress During Past Twelve Months: Until this year, the largest part of the physiology, biochemistry and psychology research programs have consisted of studies of the population of the Baltimore City Hospitals Infirmary Division. There are sampling disadvantages associated with the use of any institutionalized population. Although in most of our studies we have been able to identify serious disease and eliminate it from the group studied, the unidentified effect of lower socio-economic status of this population has undoubtedly altered the results of the psychological tests and may have altered the results of biochemical and physiological studies. Moreover, short term studies of age differences can only be conducted with the cross-sectional approach in which conventionally defined age groups are sampled at approximately the same time (within two or three years). The results of such studies cannot predict

satisfactorily the future course of physiological indices in an individual primarily because some 50 year old subjects and 80 year old subjects may have equivalent function even though they are selected from groups whose mean values are markedly different. During the past year, we have been fortunate to recruit a group of highly trained professional men who have agreed to serve now and in future years as subjects for studies carried out in this laboratory. These subjects live in the community outside of institutions and are both actively working and retired. Subjects are chosen by other subjects rather than referred by physicians or chosen by this organization. Initially, the subject is given a thorough physical examination and the results of this and any tests which may be of clinical interest are reported to his physician. Testing, which is primarily oriented toward the cardiovascular, pulmonary and renal systems, has been carried out on 34 subjects from this group whose ages range from 23 to 97 years.

d. Direction of Current Research: We will attempt to identify effects of socio-economic and educational differences between the hospital patients and the group referred to here.

An additional series of tests with emphasis on body composition and the neuromuscular system will be begun next year. About four such batteries of tests will be conducted before the present series is repeated. This will provide repeat tests every five years on the average.

Data handling will be considered in the light of the number of variables involved and the number of subjects anticipated.

2. Patient Days: None

3. Collaborators: W. W. Peter, Special Consultant
Scientists' Cliffs, Port Republic, Maryland

4. Publications and Awards:

Shock, N. W.: Some physiological aspects of aging. In: T. C. Desmond (Chairman), Good News for Later Life. New York State Joint Legis. Commit. on Probl. of Aging, Legis. Doc. No. 8, 1958, pp. 82-85.

Prepared by Arthur H. Norris
November 3, 1958

Serial No.: NHI-70

GERONTOLOGY BRANCH

Project Report

January 1958 - December 1958

1. a. Title: A study of CENTRAL-NERVOUS-SYSTEM factors in aging deficit.

In two papers given at the Conference on Planning Research on the Psychological Aspects of Aging (1955), it was suggested that the motor deficit observed in aging may be ultimately referred to changes in the central nervous system. While this is a plausible hypothesis, there is little empirical evidence to support or refute it. Moreover, the locus and nature of the postulated changes have not been specified, nor have the mechanisms by which they appear peripherally been described.

The aim of this project is to investigate motor and sensory deficit in the framework of a study designed to provide data on central-nervous-system activity. In this context, we will attack problems relative to the general topics of attention and motivation as well as those of deficit.

Working Hypotheses

1. In the absence of pathology, it is hypothesized that the sensory and motor deficits or decrements observed in aging are not independently occurring phenomena; decrement in one system is related to, and goes hand in hand with, decrement in the other.

2. Such instances of impairment are peripheral concomitants or symptoms of changes in the activity level of the Brain Stem Reticular System (BSRS). The BSRS, thus, is postulated to be the common factor behind sensory and motor deficit.

3. In non-aged subjects, changes in the activity level of the BSRS appear, on the behavioral side, as changes in what are referred to classically as attention, motivation, and affect or emotion. We postulate that fluctuations in these processes are related to BSRS activity in the same manner that sensory and motor decrements are in aged subjects, and refer to a particular level of activity of the BSRS as the Central Motive State

(CMS) of the subject. With high CMS, subjects are alert and motivated. Under conditions of very high CMS, subjects will display symptoms of heightened affect or emotion. On the other hand, with low levels of CMS, subjects would tend to be lethargic, slow responding, and would show higher sensory thresholds. We believe that aged subjects as a group will tend to fall in this category--low CMS. The lowest level of CMS on this continuum would be observed in deep sleep.

4. The activity level of the BSRS can be observed and recorded in intact human subjects by recording (electrically) the amplitude of myotatic (stretch) reflexes. Presently available neurophysiological evidence strongly supports use of this method.

5. CMS and the level of activity of the autonomic nervous system are related. The latter is related to some aspect of the electrical phenomena of the skin.

Plan of Studies

1. The initial study will be validational and will investigate the relation of the amplitude of the stretch reflex and the galvanic phenomena of the skin to conditions of sleep, rest, and activity as, for example, participation in a simple and choice reaction task. We will attempt to show that these measures vary concomitantly with gross changes in behavior (i.e., changes from sleep to rest to activity) in separate groups of aged and youthful subjects. Data from aged and non-aged groups will be compared. We expect that significant group differences in these measures will appear, with the aged group showing an over-all lower level of CMS.

It is worth mentioning in this context that the Center for the Study of Aging at Duke University recently reported that an elderly group of subjects showed decreased mean amplitudes of galvanic skin responses, as well as a decreased number of "spontaneous" galvanic-skin-response fluctuations, when compared with a young group. These findings are related to what the investigators term a decrease in C.N.S. arousal, and are found to correlate with decreased ability of the elderly group in verbal recall. We expect to corroborate and enlarge on these findings.

2. A second study will be concerned with the relation of sensory and motor deficit in aging to Central Motive State. Visual, auditory, and pain-threshold determinations will be made concurrently with recordings of stretch-reflex amplitude and the galvanic-skin phenomena. Sensory and motor-response efficiency over a two-hour period of continuous vigil will be studied through the use of a Mackworth "clock". Data will be analyzed in terms of possible relations between sensory and motor function and level of CMS.

3. A third investigation will deal with the determination of CMS in a study described elsewhere under the title of "Reaction Time and Electroencephalographic Correlates as Functions of Aging."

4. Long-range plans include studies which will be concerned with the experimental manipulation of CMS in the aged through the use of drugs.

b. Principal Investigator: Walter W. Surwillo (2/5 time)

c. Progress During Past Twelve Months: This project is still not beyond the stage of a few pilot runs with subjects. The laboratory is now nearly complete. The equipment for eliciting and recording the stretch reflex is nearly complete. Trial recordings of the galvanic phenomena (skin potentials) reveal that the temperature of the skin at the site of the electrodes is a factor in the D. C. potential observed. It will be necessary, therefore, to record skin temperature at the site of each electrode. Additional apparatus for this purpose is required. A display for the presentation of either visual or auditory stimuli in a simple and choice reaction-time situation has been completed.

Progress on this project has been slow for two reasons:

1. Adequate assistance is not available for the construction, maintenance, and repair of the electronic and mechanical instruments used in this program.

2. Adequate technical assistance is not available in the laboratory for this project. Although through necessity the investigator has instrumented this project with a view to operating with as little assistance as possible, pilot runs reveal that it is not feasible to operate alone. A fairly high level of technical assistance is required for multi-channel recording of this sort. This assistance is not presently available. Fortunately, in this situation, a new study, described elsewhere in this report (viz., the E.E.G. study), has developed to the point where it is profitably diverting a considerable amount of the investigator's time. In this new project, it has been feasible to operate more independently and proceed with the assistance available.

d. Direction of Current Research: As circumstances permit, the direction of the project will be maintained as indicated above.

2. Patient Days: Not applicable

3. Collaborators: None

4. Publications and Awards: None

Prepared by Walter W. Surwillo
October 30, 1958

Serial No.: NHI-71

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Thyroid function and age. Thyroxine degradation study.

b. Principal Investigators: Robert I. Gregerman (1/1/58 - 6/30/58) (1/2 time)
George W. Gaffney (10/15/58 - 12/31/58) (1/2 time)

Other Investigator: Marvin J. Yiengst

Technical Assistance: S. E. Crowder (3/5 time)(1/1/58 - 10/15/58)
Margaret Sellmayer (1/2 time)
Raymond Flath (1/5 time)

c. Progress During Past Twelve Months: Additional subjects have been studied with respect to the rate of disappearance of tracer doses of intravenously administered I^{131} tagged thyroxine from the blood. A total of 70 men aged 18-90 years have been tested. There is a highly significant reduction in the rate of disappearance of thyroxine from the blood.

Similar experiments were conducted in a series of old and young rats under a variety of experimental conditions. These data are being analyzed.

d. Direction of Current Research: The data will be analyzed and prepared for publication.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals provide space and all utilities.

4. Publications and Awards: None

Prepared by N. W. Shock
November 10, 1958

Serial No.: NHI-72

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Cardiovascular hemodynamics. I. Arterial performance in man.

b. Principal Investigator: Milton Landowne*

Technical Assistance: None

c. Progress During Past Twelve Months: Experimental work has been terminated.

d. Direction of Current Research: Project terminated.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards:

1. Landowne, M.: Characteristics of impact and pulse wave propagation in brachial and radial arteries. J. appl. Physiol., 12: (1), 91-97, Jan. 1958.

2. Landowne, M.: The relation between intra-arterial pressure and impact pulse wave velocity with regard to age and arteriosclerosis. J. Geront., 13: (2), 153-161, April 1958.

Prepared by W. W. Shock
October 31, 1958

* No longer on staff.

Serial No.: NHI-73

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Cardiovascular Hemodynamics. II. Cardiac performance in man.

b. Principal Investigators: Milton Landowne
Joseph Falzone
Theodore Reiff

c. Project terminated.

Prepared by N. W. Shock
October 31, 1958

Serial No.: NHI-74

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Studies on the effects of temperature on the aging process. I. Drosophila studies.

   b. Principal Investigator: Bernard L. Strehler (20% of time)

   Technical Assistance: M. Susanne Herman (60% of time)

   c. Progress During Past Twelve Months: A great quantity of data on the longevity of Drosophila at different temperatures has been developed according to the methods outlined in last year's report.

   At present, incomplete evidence indicates that short exposure to higher temperatures (up to 36° C.) does not appreciably change mortality rates, but that there is a permanent effect induced by exposure to temperatures above 38° C.

   d. Direction of Current Research: Various combinations of duration and intensity of exposure are being carried out.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Bernard L. Strehler
October 31, 1958

Serial No.: NHI-75

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Care of patients housed on Ward B-2 (60 beds) who participate in studies performed in this unit. In addition, all males acceptable for admission to Baltimore City Hospitals Infirmary receive medical screening and treatment.

   b. Principal Investigator: Richard L. Myers (1/1/58 - 7/1/58)
   Jerome Lowenstein (7/1/58 - 12/31/58) (3/4 time)

   Other Investigator: Joseph A. Falzone (1/4 time)

   Technical Assistance: Baltimore City Hospitals Clinical Laboratories

   c. Progress During Past Twelve Months: Assigned July 1, 1958 through December 31, 1958

   d. Direction of Current Research: Stated above.

2. Patient Days: Not applicable.

3. Collaborators: Baltimore City Hospitals staff and clinical facilities.

4. Publications and Awards: None

Prepared by Jerome Lowenstein
October 31, 1958

Serial No.: NHI-76

GERONTOLOGY BRANCH

Project Report

August 18, 1958 - December 31, 1958

1. a. Title: Theoretical study of distribution of metabolites around capillaries.

b. Principal Investigator: Jacob J. Blum

Other Investigators: None

Objectives: To develop equations for predicting the distribution of metabolites in the tissue surrounding a capillary. The problem can best be visualized as follows: Arterial blood, flowing at a constant velocity, v, and containing a metabolite of concentration C_a, enters a capillary of uniform radius R_1. As the blood (or other perfusing fluid) flows through the capillary, the metabolite passes out through the membrane of permeability H and diffuses into the tissue (diffusion constant, D), where it is consumed by the tissue in either a zero order, a first order, or, in the general case, a Michaelis-Menten type of metabolic reaction. It is desired to know the concentration of the metabolite at any point inside or outside the capillary.

Methods Employed: The methods of mathematical physics. In later stages of this work it may be desirable to compute various concentration profiles as a function of the permeability, the diffusion constant, the velocity of blood flow, the capillary radius, etc. on a high speed computer.

Major Findings: Equations have already been derived for first order and zero order chemical reactions in the tissues.

Significance to the Program of the Institute: If it were possible to predict the concentration profiles of a substrate such as oxygen or glucose around the capillaries, then one could investigate the variation of capillary permeability and tissue diffusion constants with old age or with any other physiologically interesting variable (exercise diseases of various sorts, diet, etc.).

Proposed Course of Project: It is hoped to: (1) investigate non-stationary states, (2) study the effects of diffusion in the axial direction to see whether it is justified to neglect these effects and (3) develop approximate equations for treating the case when the tissue around the capillary consumes the substrate according to a non-linear rate.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Jacob J. Blum
October 31, 1958

Serial No.: NHI-77

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Studies on relationships between development and aging. I. Developmental correlations between the eye and the nervous system in the mosquito, Aedes aegypti.

b. Principal Investigator: Dietrich Bodenstein (5%)

Other Investigator: Richard White
(Washington University, St. Louis, Mo.)
(Summer Fellowship)

c. Progress During Past Twelve Months: This project was begun in June 1958. The compound eyes of insects are intimately connected with the cerebral nervous mass known as the optic lobes. Each optic lobe is made up of three optic glomeruli. These structures, with the possible exception of one, arise during metamorphosis by the transformation of the larval optic ganglion. The problem under investigation is to ascertain whether a reduction of the peripheral field (load) in early larval life has any effect on the central nervous system. To this end a micro-cautery was designed by which small portions of the peripheral field, i.e., the rudimentary eye, could be destroyed. A large number of operations (cauterizations) were performed on animals in different stages of development and marked eye defects in the adult mosquito were thus obtained. In order to study the possible effects of the experimentally produced eye defect on the optic ganglion, special nerve staining methods were developed.

d. Direction of Current Research: This material is now ready for histological study. Mr. White has returned to Washington University where he will continue these investigations.

2. Patient Days: None

3. Collaborators: None

4. Publications and Awards: None

Prepared by Dietrich Bodenstein
October 30, 1958

Serial No.: NHI-78

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Studies on relationships between development and aging: II. Humoral control of the accessory sex glands in the cockroach, Periplaneta americana.

b. Principal Investigator: Dietrich Bodenstein (10%)

Other Investigator: Isabelle Sprague
(Mt. Holyoke College - National Science Foundation Fellowship)

c. Progress During Past Twelve Months: This project was begun about September 1958.

d. Direction of Current Research: This problem has been attacked by the use of transplantation, extirpation and grafting techniques. The secretory activity of the secondary female sex glands is controlled by the juvenile hormone produced by the corpus allatum. Thus, by implanting the non-functioning gland of a teneral adult female into the body cavity of a series of nymphs of known age, one can assay the allatum hormone level in the host body. This study, already quite far advanced, indicates that the hormone level differs in animals of different age.

Another related problem under investigation is to determine at what stage during the post-embryonic development of the insect the sex glands are competent to respond to humoral influences. This is being studied by transplanting glands of different age into numphal and adult hosts, i.e., into animals of different humoral environments. By the removal of different glands of internal secretion we are also able to alter the humoral environment, and to use such altered animals as hosts for the sex gland transplants.

2. Patient Days: None

3. Collaborators: None

4. Publications and Awards: None

Prepared by Dietrich Bodenstein
October 30, 1958

Serial No.: NHI-79

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Studies on relationships between development and aging: III. Humoral control of ovary development in the cockroach, Periplaneta americana.

b. Principal Investigator: Dietrich Bodenstein (35%)

Technical Assistance: Wilma Gabbay (35%)

c. Progress During Past Twelve Months: This is a project begun about January 1958. This study was designed to elucidate the hormonal regulation of ovarian development. It includes a comparison of the developmental behavior of the ovarioles in hosts of different age. We have found striking cytological differences in the cells of the various epithelial regions of the ovariole. It is known that the juvenile hormone is necessary for ovary maturation but it is not known whether the observed cytological variations are under the same humoral control.

d. Direction of Current Research: This problem is being investigated by the transplantation of different parts of the ovariole into pregnant hosts. In the course of these experiments we have been led to believe that the corpus luteum plays an important role in egg maturation. The humoral cycle controlling the development of the egg appears much more complex than originally visualized and the possible involvement of the corpus luteum in this cycle is of the greatest significance.

2. Patient Days: None

3. Collaborators: None

4. Publications and Awards: None

Prepared by Dietrich Bodenstein
October 30, 1958

Serial No.: NHI-80

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Studies on relationships between development and aging: V. Nerve and muscle atrophy in the cockroach, Periplaneta americana.

b. Principal Investigator: Dietrich Bodenstein (5%)

Technical Assistance: Wilma Gabbay (5%)
Joanne Delp (25%)

c. Progress During Past Twelve Months: This project was begun in June 1958. Certain coxal muscles of adult cockroaches can be rendered functionless by the removal of the distal part of the leg. In these cases it has been observed that the nerves innervating these muscles suffer atrophic changes. This effect might be due to a reduction in the diameter of the individual nerve fibers or a loss of fibers. A corollary to this problem is whether the inactive muscles undergo degenerative changes and, if so, what sort of changes. There is some evidence of muscle change correlated with age but beyond this we have no further information.

d. Direction of Current Research: A large series of experiments have been set up to investigate this. This is a long-range project since it takes months before the effects occur. We intend to sacrifice these animals at different time intervals and study these changes histologically and histochemically in detail.

2. Patient Days: None

3. Collaborators: None

4. Publications and Awards:

Bodenstein, Dietrich (in collaboration with V. Detheir): Hunger in the blowfly. Zeit f. Tierpsychologie, 15: 129-140, 1958.

Elected member of the National Academy of Sciences.

Prepared by Dietrich Bodenstein
October 30, 1958

GERONTOLOGY BRANCH

Project Report

January 1958 - December 1958

1. a. Title: Structure of hemoproteins.

b. Principal Investigator: Gunther L. Eichhorn (1/2 time)

Technical Assistance: Albert Osbahr (1/2 time)

Objectives:
a. To elucidate the nature of the relationship between protein and prosthetic group in such compounds as hemoglobin, cytochrome-C, etc.

b. To evaluate the relationship between the structures of these compounds and their rotatory dispersion curves.

Methods Employed: Samples of various biologically important hemoproteins are prepared, and their absorption spectra and rotatory dispersion curves are determined. If the rotatory dispersion is anomalous in regions where the heme absorbs, the heme group must be an asymmetric center. It is then possible to subject the molecules to various chemical stresses, and to determine the effect upon the asymmetric center.

Major Findings: The heme group in hematin is not asymmetric, but it becomes a center of asymmetry in cytochrome-C, hemoglobin, and myoglobin. A close fit between absorption maxima and anomalous dispersion is found for reduced hemoglobin, oxyhemoglobin, and methemoglobin.

When hemoglobin is cleaved into half-molecules, dispersion studies indicate that the heme asymmetry is lost. This indicates that the four heme groups in the hemoglobin molecule are so placed that both sides of the heme group are identical in the half-molecule.

Significance of the Program to the Institute: The compounds under investigation are of great importance in the chemistry of blood and the chemistry of living cells. The Gerontology Branch of the Heart Institute is interested in the functional changes that accompany the aging process; a knowledge of the structure of the important cellular constituents is of prime importance in the understanding of biological functions as well as of changes in these functions.

Direction of Current Research: The hemoglobin molecule will be dissected by various chemical techniques, and the effect of each dissection upon the heme asymmetry will be determined. It is hoped in this way to obtain an accurate placement of the heme within hemoglobin.

The mechanism of hemoglobin denaturation will be followed by rotatory dispersion techniques.

Similar studies will be carried out on catalase, peroxidase, chlorophyll, and vitamin B_{12}.

Publications and Awards:

Eichhorn, G. L., and J. F. Cairns: Rotatory dispersion of cytochrome-C. Nature, 181, 994, 1953.

Prepared by Gunther L. Eichhorn
October 31, 1958

Serial No.: NHI-82

GERONTOLOGY BRANCH

Project Report

January 1958 - December 1958

1. a. Title: Age changes in the chemical composition of various tissues of the rat.

Objectives: The objectives of this project are to investigate age changes in chemical composition of tissues. The aim of specific investigations now in progress is a twofold study to determine (1) the effect of age on the serum proteins of rats, and (2) whether such age differences may be explained on the basis of serum protein synthesis.

Methods: Standard methods of chemical analysis are used for the determination of serum protein and albumin. These measurements will be done on animals aged 2, 12 and 24 months. In addition, animals of these same age groups will be (1) subjected to 21 days of protein-free feeding and (2) 3 day or 7 day repletion with the standard laboratory diet.

b. Principal Investigator: M. J. Yiengst (1/4 time)

Other Investigator: Charles H. Barrows

Technical Assistance: Theresa Caryk (1/4 time)

c. Progress During Past Twelve Months: Thus far this experiment has been done on 34 rats aged 24 months. There were no changes of any significance in serum globulin levels during the experiment. However, the albumin concentration decreased markedly during the 3 week depletion period followed by a return to control values after 3 days of repletion and a small overshoot after 7 days of repletion.

d. Direction of Current Research: The procedure, as given above, will be extended to include 40 animals in each of the respective age groups.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Marvin J. Yiengst
November 1, 1958

GERONTOLOGY BRANCH

Project Report

January 1958 - December 1958

1. a. Title: Age studies of cell particulates and fractions.

   b. Principal Investigator: Joseph A. Falzone (1/2 time)

   Other Investigators: Charles Barrows
   Marvin J. Yiengst
   Nathan W. Shock

   Technical Assistance: Theresa Caryk (1/4 time)
   Lois Roeder (1/4 time)

Objectives: This project is designed to measure age changes in the morphology, chemistry, and function of separable protoplasmic particles. The working hypothesis of these studies is that overall cell function may be well maintained until the moment of cell death by virtue of compensatory adjustments in the activities of its parts.

Methods Employed: Animals used will generally be the same as for Project No. 153; i.e., young adult and old rats aged 12 and 24 months respectively. The basic technique is that of differential centrifugation of tissue homogenates at 0°-4° C using an ordinary or ultracentrifuge depending upon particle size. Other techniques required by special problems are indicated below.

c. Progress During Past Twelve Months: Nuclear studies. Equations have been derived which predict the theoretical density gradient produced by our machine. The measured gradient (last report) agrees reasonably well with the predicted gradient. Experimental runs on an additional 8 rats have been completed with results similar to those reported previously, i.e., a clean separation of diploid from tetraploid liver nuclei. The experimental work for this study is thus considered complete and a manuscript is in preparation.

The age comparison of mean DNA per nucleus in rat liver (n = 38) was reported previously. We have also found that in sections average volume of hepatic nuclei or incidence of giant nuclei does not change significantly with age. The occurrence of giant nuclei is not frequent enough to significantly alter mean DNA per nucleus results. Calculated mean DNA per hepatic nucleus (from the differential counts and DNA value for stromal nuclei) is also not significantly different with age or sex.

Mitochondrial studies. For the extremely interesting metabolic findings, see Dr. Barrows' report. Our preparative technique is virtually identical to that used by Weinbach and Garbus (personal communication from Dr. Weinbach) so that differences in results cannot be explained on this basis.

RNA study. RNA analyses on fractions from the last 10 rats have been completed with results essentially the same as those described previously. Statistical analysis of these data is not complete so we prefer not to make any summary statement at this time.

No further extensions of cell fractionation techniques have been made since last report.

d. Direction of Current Research: Completion of mitochondrial study. This will probably involve oxidative phosphorylation runs on another group of 40 animals, probably of one sex, with age levels intermediate between those already studied.

Application of routine histological methods to rat tissues. This has several purposes.

1. Qualitative description of tissues used for metabolic studies and to supplement gross autopsy findings on rats dying spontaneously.

2. Application to other studies.

3. To obtain quantitative data to be correlated with chemical findings (in very selected problems, i.e., as we have done with nuclear volume and DNA content).

Most of the equipment for this has been obtained.

Extension of cell fractionation techniques as described previously, with emphasis upon isolation of whole cells of a single type for metabolic studies. These needs are not answered by tissue culture because of the demonstrated metabolic alterations produced by this environment.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards:

1. Falzone, J. A., Jr., C. H. Barrows, Jr., and N. W. Shock: Age and polyploidy of rat liver nuclei as measured by volume and DNA content. J. Geront. (In press).

Prepared by Joseph A. Falzone, Jr.
November 5, 1958

GERONTOLOGY BRANCH

Project Report

January 1958 - December 1958

1. a. **Title: Pulmonary physiology as related to age.**

The goals and aims of this project are to describe age changes in pulmonary function. These studies involve measurements of the volumes of the lung compartments and the functional capacity of the pulmonary system, including the mechanical aspects of the bellows function and the responsiveness of the pulmonary system to experimental stimulation and displacement.

In addition to the standard methods of measuring lung volumes, a helium washout technique has been developed to give estimates of functional volumes as well. An attempt will be made to relate functional measurements to anatomical measurements made directly on the chest and to roentgenographic measurements. In addition, the laboratory measurements of pulmonary function are being compared with the responses to exercise and with clinical estimates of pulmonary and work performance limitations of older subjects.

This is a continuing program. Not only will subjects of different ages be compared, but individuals who are available will be remeasured at intervals of three to seven years.

b. Principal Investigator: Arthur H. Norris (1/4 time)

Other Investigators: Joseph A. Falzone, Jr.
David A. Oursler

Technical Assistance: John B. Melvin (1/3 time)
Mae F. Moody (1/3 time)

c. Progress During Past Twelve Months: Measurements of height and spinal curvature have been completed for 92 subjects from 20 to 89 years old who have had satisfactory lateral chest x-rays as well as pulmonary function tests. Thoracic height was measured from the middle of the top of the third thoracic vertebra to the middle of the bottom of the twelfth thoracic vertebra and spinal curvature was measured as the perpendicular distance from the front (ventral) side of the eighth thoracic vertebra to the line which measured thoracic

height. The regression of thoracic height on age was 0.3 cm. in 10 years while the distance between the front of T8 and the line between T3 and T12 increased 0.1 cm. in 10 years. Geometric comparison of these two measurements indicated that the reduction of thoracic height was attributable to spinal curvature rather than possible intervertebral disc compression.

Preliminary experimental measurements of the oxygen cost of lung ventilation have been carried out and are being evaluated. Pulmonary function tests are being performed on a group of subjects of various ages whose socio-economic level is higher than those previously tested under this program and who have agreed to be available for retest.

d. Direction of Current Research: Methods for measuring the oxygen cost of lung ventilation will be evaluated. A satisfactory method for measuring the oxygen cost of ventilation will permit correction for possible age differences in this function and thereby provide better estimates of mechanical efficiency. Studies of age changes in the elastic properties of the intact lung will be attempted. Since aging is accompanied by loss of tissue elasticity, measurements on the lung may afford a useful index of physiologic age.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Arthur H. Norris
November 3, 1958

Serial No.: NHI-85

GERONTOLOGY BRANCH

Project Report

January 1958 - December 1958

1. a. Title: Age difference in body size and composition.
This project is designed to describe age differences in body size and composition, to compare various size and composition measures made concurrently in individual subjects, and to examine the relationship of these age differences and comparisons to physiological responses.

Height and weight data will be obtained by the usual anthropometric methods. The volume of the body will be measured by its displacement of helium in a closed chamber. The density of the body will be calculated from these measures after correction for total skeletal mass estimated from roentgenographic films of the femur and humerus. Body fat will also be estimated from skinfold thickness and roentgenographic techniques. Estimates of lean body mass will include (1) basal metabolic rate determinations made with standard open circuit methods and (2) body water and fluid distribution determinations made from the distribution curves of injected antipyrene and sodium thiocyanate.

This is a continuing program. These studies will be carried out in people of different ages and in the same people as they become older.

b. Principal Investigators: Arthur H. Norris (1/4 time)
Theodore H. Lundy (Appointed July 1, 1958)

Other Investigators: Nathan W. Shock
Marvin J. Yiengst

Technical Assistance: Constantine J. Manaras

c. Progress During Past Twelve Months: Improvements have been made in the instrumentation and technique of measuring body volume by helium displacement which permit reproducibility of 0.3% for either humans or standard volumes.

Body composition measurements have been performed on 30 subjects of various ages who have agreed to be available for

retest. Twenty-one of these have total body density values which are in a reasonable range (0.98 to 1.050). These data are being evaluated and compared to the other body composition estimates.

d. Direction of Current Research: An integrated measurement program which includes selected indices of body size and composition will be continued. Some techniques are provided through cooperation of investigators outside the Public Health Service while others are standardized procedures used in this laboratory. Data are available for comparison with other physiological data which may be collected on subjects of these studies. Moreover, subjects whose size (or composition) varies widely from mean values will be selected for study, and experimental and therapeutic displacements of body composition may be attempted.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

Dr. Stanley M. Garn, Fels Research Institute, Antioch College, Yellow Springs, Ohio

Dr. Harald Schraer, The Bone Density Research and Evaluation Center, Pennsylvania State University, University Park, Pennsylvania

Dr. Paul Baker, The Bone Density Research and Evaluation Center, Pennsylvania State University, University Park, Pennsylvania

Dr. Saul P. Baker, Department of Medicine, Chicago Medical School, Chicago, Illinois

4. Publications and Awards: None

Prepared by Arthur H. Norris
November 3, 1958

Serial No.: NHI-86

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Osmotic Homeostasis

b. Principal Investigator: Theodore Reiff (1/1/58 - 6/30/58)

Other Investigators: Marvin J. Yiengst
N. W. Shock

Technical Assistance: Raymond Flath

c. Progress During Past Twelve Months: A colloid osmometer has been described with the following advantageous characteristics:

(1) It is entirely automatic--i.e., no balancing of oncotic pressure with an externally applied pressure is necessary.

(2) A complete pressure-time tracing of the approach to equilibrium is obtained so that the course may be followed during this actual run.

(3) Serial determinations may be easily and very rapidly performed.

(4) The instrument is capable of semi-micro determinations on volumes from 0.5 to 0.1 $cm.^3$. By making a smaller solution chamber, the volume of solution needed can be made correspondingly less.

Measurements of the rate of adjustment of oncotic pressure of the blood, following the intravenous administration of serum albumin has been measured in a selected group of subjects.

d. Direction of Current Research: This specific project has been completed.

2. Patient Days: None.

3. Collaborators: Baltimore City Hospitals.

4. Publications and Awards:
Reiff, T. R., and M. J. Yiengst: A rapid automatic semi-micro colloid osmometer. J. Lab. clin. Med., (in press).

Prepared by N. W. Shock
October 31, 1958

GERONTOLOGY BRANCH

Project Report

August 18, 1958 - December 31, 1958

1. a. Title: The role of sulfhydryl groups in muscle action.

b. Principal Investigator: Jacob J. Blum

Other Investigators: None

Objectives: It is known that the muscle protein myosin, which catalyzes the enzymatic hydrolysis of adenosine triphosphate and of its analogues (UTP, CTP, GTP, ITP) has many sulfhydryl groups which are important in the enzymatic and in the contractile processes. By the use of certain SH group blocking agents such as parachloromercuribenzoate (PCMB), it is possible to accelerate and/or inhibit the rate of hydrolysis of ATP and its analogues. By detailed study of the kinetics, it is hoped to deduce the contribution of the aromatic ring to the binding of the substrate to the enzymatic site. Such information will be of value not only in understanding the mechanism of action of myosin as an enzyme, but may also permit insight into the nature of the transduction of the chemical energy of ATP into mechanical energy. By comparing the effect of PCMB on the contractile responses of the muscular system when ATP, ITP, etc. are used as substrates, it will be possible to correlate the enzymatic effects of SH groups and of the substrate aromatic ring with the mechanical changes. The contractile effects will be studied on muscle models of varying complexity ranging from purified actin-myosin mixtures to glycerol treated psoas fibers. Since it is highly probable that the SH groups in the muscle protein actin are intimately involved in the contractile process, it will also be necessary to investigate the effects of PCMB on the interaction between myosin and actin.

Methods Employed: Enzymatic rates will be measured by the Fiske-Subbarow method and by pH measurements in unbuffered solutions. Sulfydryl groups will be estimated by spectrophotometric procedures. Contractile studies will be done both isotonically and isometrically on glycerol treated psoas fibers. Changes in length and/or aggregation of actomyosin in solution will be examined primarily by light scattering and superprecipitation studies.

Major Findings: None. Work will begin on this project when the laboratories are ready.

Significance of this Research to the Program of the Institute: Sulfydryl groups play an important role in the structure and function of many proteins and any further knowledge of their role in the conversion of chemical to mechanical energy will be of value in many areas of biology.

Proposed Course of Project: The interaction of ATP, ITP, etc. with myosin affects not only the substrate (which is hydrolysed) but also the enzyme. The different analogues affect the heat stability of the enzyme differently and it is probable that SH groups are involved in determining the degree of heat stability that a given substrate can confer on the protein. This project will begin with a detailed study of the reciprocal effects of PCMB and of ATP and its analogues on the thermal stability of myosin and a comparison of these effects on thermal stability with the enzymatic activity of myosin under the same conditions.

2. Patient Days: None.

3. Collaborators: Baltimore City Hospitals.

4. Publications and Awards: None.

Prepared by Jacob J. Blum
October 31, 1958

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Studies on the comparative physiology of senescence: Campanularia regression.

b. Principal Investigator: Bernard L. Strehler (10% of time)

Other Investigators: Sears Crowell, Marine Biological Laboratories and Indiana University
Matthew Pollack, Baltimore City Hospitals
Robert Kohn, Rockefeller Institute
Charles Wyttenbach, Carnegie Institute

Technical Assistance: Malcolm Gee (10% of time)

c. Progress During Past Twelve Months: This research, which was designed to elucidate the chemical mechanisms underlying, and the physiological significance of the systematic regression of the hydranths of the colonial hydroid, Campanularia, was carried out at the Marine Biological Laboratories, Woods Hole, Massachusetts.

It was shown that the older hydranths are comparable to young ones up to the onset of regression in their capacity to (1) catch food (artemia) and (2) digest and transmit food to the other members of the colony.

It was also shown that the concentration of ATP of hydranths drops continually during their aging process and reaches vanishing concentrations (by extrapolation) at the time of regression. Thus it may be that the limiting factor for hydranth persistence is the available pool of purine nucleotides.

These combined studies suggest that hydranths do not undergo degeneration because of a loss of capacity to function normally. Rather they suggest that senescence may have an adaptive value to the colony by forcing a redistribution of feeding bodies to points of greatest efficiency (the periphery) on a regular basis.

d. Direction of Current Research: We are attempting to culture these animals in the laboratory in order to investigate further the mechanisms of their growth, senescence and regeneration cycle.

2. Patient Days: None

3. Collaborator: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Bernard L. Strehler
October 31, 1958

Serial No.: NHI-89

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Studies on changes in localization and/or concentration of molecular populations during aging.

   b. Principal Investigator: Bernard L. Strehler (10% of time)

      Technical Assistance: Malcolm Gee (20% of time)

   c. Progress During Past Twelve Months:
      (1) Paper chromatography of fluorescent amino acids - no progress.

      (2) Electrophoresis - Starch gel electrophoresis of various organ extracts followed by histochemical localization has been successfully undertaken.

      (3) ATP assay vs. age - See report on Campanularia studies.

   d. Direction of Current Research: This program area is in temporary abeyance because of the intensive effort on the more promising areas of activity.

2. Patient Days: None.

3. Collaborators: Baltimore City Hospitals.

4. Publications and Awards: None.

Prepared by Bernard L. Strehler
October 31, 1958

Serial No.: NHI-90

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: The effects of aging on the developmental capacity of organ discs in the fruit-fly, Drosophila.

b. Principal Investigator: Dietrich Bodenstein (10%)

Technical Assistance: Wilma Gabbay (25%)

c. Progress During Past Twelve Months: This is a new project begun about June 1958. Our Drosophila cultures for these experiments are now ready, and some preliminary control experiments have been performed.

d. Direction of Current Research: Organ discs of Drosophila increase in size during larval life. Thus, during this period, only growth takes place. They differentiate into the imaginal structure during pupal life, a span of about three days. We intend to investigate whether aging, without allowing for growth and differentiation, has any effect on the developmental capacities of these organ discs. Growth and differentiation of these discs are controlled by hormones. In the absence of these hormones, these processes cannot occur. By culturing the larval discs in an adult host, no growth or differentiation occurs because the adult environment is hormone-free. These discs can be left in the adult fly for up to fourteen days (limit of life of host); they may then be taken out and retransplanted into a second host where they can remain for the same length of time. This procedure can be repeated and the discs aged for as long a period as one wishes. Different age discs can be transplanted back into a larval host where they will develop to imaginal completion in synchrony with the host organs. The developmental capacity of the aged discs can be evaluated by the structural patterns differentiated.

2. Patient Days: None.

3. Collaborators: None.

4. Publications and Awards:
Bodenstein, Dietrich: Contributions to the problem of eye pigmentation in insects: Studied by means of intergeneric organ transplantations in Diptera. Publication of Smithsonian Institution (in press).

Prepared by Dietrich Bodenstein
October 30, 1958

GERONTOLOGY BRANCH

Project Report

September 1, 1958 - December 31, 1958

1. a. Title: Studies on the mechanism of oxidative decarboxylation of α-keto acids.

   b. Principal Investigator: D. Rao Sanadi (1/2 time)

      Technical Assistance: Robert L. Searls

   c. Progress During Past Four Months: This is a new project initiated in October 1958.

   d. Direction of Current Research: Previous work has shown that the oxidation of α-ketoglutarate catalyzed by the dehydrogenase from heart muscle probably occurs in four sequential steps. The enzyme is a complex of unusually high molecular weight (2 million) containing diphosphothiamine and thioctic acid in tightly bound forms. Attempts will be made to disrupt the complex by digestion with trypsin and to devise assays for the individual surviving components. This would enable fractionation of the subunits of the complex. The ultimate aim is to isolate each of the enzymatic units and reconstitute them to obtain the complete oxidation.

2. Patient Days: None.

3. Collaborators: Baltimore City Hospitals.

4. Publications and Awards: None.

Prepared by D. Rao Sanadi
October 27, 1958

Serial No.: NHI-92

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Studies on relationship between development and aging: IV. Studies on leg regeneration in the cockroach, Periplaneta americana.

b. Principal Investigator: Dietrich Bodenstein (35%)

Technical Assistance: Wilma Gabbay (35%)
Joanne Delp (75%)

c. Progress During Past Twelve Months: This is a new project begun about January 1958. The study of regeneration offers a unique opportunity to investigate tissues in different stages of differentiation. The mature insect has lost its powers of regeneration, but by appropriate hormone application, regenerative events can again be initiated. It has been found that tissues from younger animals respond to the humoral stimulus with greater ease than those of older animals. The present investigations are concerned with the state of determination of the different parts of the regenerate and its relation to humoral and nervous influences. These problems have been attacked by following the development of isolated parts of the regenerate in vivo. Young isolates are apparently able to regulate to a considerable extent. The regulative capacity of older regenerates is much more limited.

d. Direction of Current Research: These isolates apparently do not contain nerves. Since nerves are supposedly necessary for regeneration, the isolates are currently being examined for the presence of nerves. Attempts are also being made to produce nerveless regenerates by transplanting legs heterotopically. The relation between innervation and muscle formation is an important problem, and our material is ideally suited for such investigation. It is now being examined histologically.

2. Patient Days: None

3. Collaborators: None

4. Publications and Awards:
1. Bodenstein, Dietrich: The role of hormones in the regeneration of insect organs. Scientia (in press).

Prepared by Dietrich Bodenstein
October 30, 1958

Serial No.: NHI-93

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Metabolism and Endocrinology. Thyroidal radio-iodide uptake and serum protein bound iodine as influenced by age.

b. Principal Investigators: George W. Gaffney (10/15/58 - 12/31/58)
Robert I. Gregerman (1/1/58 - 6/30/58)
(1/4 time)

Other Investigators: N. W. Shock
Marvin J. Yiengst

Technical Assistance: S. E. Crowder (1/1/58 - 10/15/58)
(1/5 time)

c. Progress During Past Twelve Months: The data on I^{131} uptake by the thyroid gland in 131 subjects aged 41-94 years have been subjected to statistical analysis. No significant change in the uptake of I^{131} by the thyroid gland was found with increasing age. No significant alterations in the level of protein bound iodine in the circulating blood was found with increasing age. It is concluded that the thyroid gland of the healthy old man makes available to him enough thyroid hormone to serve his needs under conditions of ordinary daily activity.

Administration of TSH to aged males failed to demonstrate any impairment of metabolic responses to the hormone.

d. Direction of Current Research: Work on the specific project will be concluded with the preparation of appropriate manuscripts.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals provides space and all utilities.

4. Publications and Awards:

1. Watkin, D. M.: The assessment of protein nutrition in aged man. Ann. N. Y. Acad. Sci., 69: (Art. 5), 902-915, Jan. 10, 1958.

2. Gaffney, G. W., D. M. Watkin, and B. F. Chow: Vitamin B_{12} Absorption: Relationship between oral administration and urinary excretion of cobalt^{60}-labeled cyanocobalamin following a parenteral dose. Study of doses of 2 to 250 micrograms in 148 apparently healthy men of ages 20-92. J. Lab. clin. Med., (in press).

3. Baker, S. P., G. W. Gaffney, N. W. Shock, and M. Landowne: Physiological responses of five middle-aged and elderly men to repeated administration of thyroid stimulating hormone (thyrotropin; TSH). J. Geront., (in press).

Prepared by George W. Gaffney
November 10, 1958

Serial No.: NHI-94

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Studies on optical properties of metabolizing systems.

   b. Principal Investigator: Bernard L. Strehler (20% of time)

   Technical Assistance: M. Susanne Herman (15% of time)

   c. Progress During Past Twelve Months: Considerable advances have been made in this area: (1) The transmission change induced in luminous bacteria has definitely been shown to be due to a change in scatter rather than absorption. (2) The scatter is in all probability not metallic because it shows no strong depolarizing effect on polarized light. (3) The scattering change is closely tied to the metabolic condition of the bacteria and is paralleled by an inhibition of their ability to luminesce. (4) An adaptation of a light integrator has been developed for the Cary Model 14 Spectrophotometer which permits the measurement of the absorption spectra of scattering biological materials with simplicity, proficiency and accuracy. (5) The spectral changes accompanying active respiration by luminous bacteria have been examined.

   d. Direction of Current Research: This phase of the work is essentially completed and is being prepared for publication.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Bernard L. Strehler
October 31, 1958

Serial No.: NHI-95

GERONTOLOGY BRANCH

Project Report

January 1, 1958 - December 31, 1958

1. a. Title: Studies on the differential fluorescent. Labelling of pituitary cells and granules obtained from them.

   b. Principal Investigator: Bernard L. Strehler

   c. Progress During Past Twelve Months: This project was not actively pursued this year because of other interests.

   d. Direction of Current Research: This project is inactive at the present time but will be resumed later.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Bernard L. Strehler
October 31, 1958

GERONTOLOGY BRANCH

Project Report

January 1958 - December 1958

1. a. Title: Studies on the effect of temperature on the aging process. II. Bacterial and enzyme "aging" studies.

b. Principal Investigator: B. L. Strehler (5%)

Technical Assistance: M. Susanne Herman (10%)

c. Progress During Past Twelve Months: Because other areas of research have demanded our attention there has been little additional work on this project this year. It has become clear that the thermal effects are not simple kinetically and rather than invest a large fraction of time it was decided to invest our effort on more directly promising lines of research. Although thermal shock does not appear to produce permanent damage to all luminous bacteria surviving a shock, there is a definite sensitization of the bacteria to thermal death or to destruction of the luminescent system for a short period after exposure if the exposure is not extreme. This is deduced from the fact that there is a greater rate of loss of luminescence for some time after thermal shock than in its absence. The rate then slowly approaches the original rate.

d. Direction of Current Research: The luminescent system of luminous bacteria is being purified in order to determine:

1. Whether generally reproducible and kinetically simpler effects can be obtained with an extracted system.

2. Whether the enzymes involved are destroyed in a stepwise manner during thermal denaturation.

We will attempt to crystallize the luminescent enzyme, luciferase, in order to give an absolutely uniform material whose kinetic behavior can be measured with ease.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Bernard L. Strehler
November 1, 1958

GERONTOLOGY BRANCH

Project Report

January 1958 - December 1958

1. a. Title: Verbal performance as a function of aging.

Goal: The study of verbal skill has been somewhat neglected relative to other skills; e.g., motor. It is our hope that this neglect will be modified; and it is our belief that the study of verbal performance will lead to significant findings regarding age differences in a variety of psychological processes. We intend, therefore, to study the relationships of selected parameters of verbal behavior to aging, and to eventually relate these parameters to other psychological processes such as communication between and with the aged. Two parameters of verbal behavior have been selected for preliminary study: encoding ability, and meanings of age-relevant concepts.

i. With respect to encoding the following hypothesis will be tested: Errors and time of encoding increase as a function of age. This hypothesis is simply an extension of what has been found with other skills and performances. We wish to see if these findings generalize to verbal performance.

ii. With respect to meanings the study will be descriptive; i.e., we will simply describe differences in concept meanings which exist to a significant degree between age groups.

Method: Hypothesis i. The original method for testing this hypothesis, due to experience during the year, has been changed since the last annual report to the following extent. The use of the Flesch count has been dropped and we are using word lists which (1) vary in length (from 10 to 25 words), and (2) vary in degree to which they approximate meaningful English language sequences. The material is repeated back to the investigator and recorded on a tape for analysis. Errors of various kinds will be defined and measured. Techniques are being developed for measuring time consumed by pauses (time between words) and by verbalization (time within words). Errors and time will be related to age.

ii. The semantic differential will be employed to measure the meanings of certain concepts. Examples of possible concepts are DEATH, OLD MAN, ADOLESCENT, etc. Concepts need to be found from the literature and from discussions between the principal investigators. These concepts will be rated on seven-point scales defined by adjective-opposites; e.g., good-bad, active-passive, strong-weak. These scales will

be chosen on the basis of extensive research already done by one of the principal investigators at the University of Illinois.
Date of commencement: October 1, 1957.

b. Principal Investigators: George J. Suci (1/4 time)
Melvin D. Davidoff (1/4 time)

Technical Assistance: Jesse Yaffa (1/3 time since September)

c. Progress During Past Twelve Months:
i. Twelve subjects in each of age groups, under 40, 40-59, 60 and over, have been run. Twelve more are needed in each age group. After preliminary testing of 15 scales and 5 concepts, the semantic differential form was changed to 11 scales and 6 concepts. Concepts are: 17 year old, 25 year old, 45 year old, 65 year old, 80 year old person, and "person your own age". From among local subjects and a sample of young people from Lititz, Pennsylvania we were able to match 35 young and 35 old people with respect to education (all with some High School education). These data were analyzed with respect to judgmental scales. Essentially identical factor structures of scale relationships were found in young and old. Two main factors are Evaluation (good-bad) and Activity (active-passive) which was akin to old-young judgments.

d. Direction of Current Research:
i. Subjects will continue to be run as indicated above.
ii. Further analysis will aim at assessing the discrimination between concepts made by these groups on these factors.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals and Baltimore Golden Age Clubs.

4. Publications and Awards: None

Prepared by Melvin D. Davidoff
October 30, 1958

Serial No.: NHI-98

GERONTOLOGY BRANCH

Project Report

January 1958 - December 1958

1. a. Title: Reaction time and electroencephalographic correlates as functions of aging.

Purpose: To investigate age decrement in simple reaction time with reference to age changes in the alpha frequency of the electroencephalogram. Our working hypothesis is derived from the observations (in the literature) that (1) simple reaction time increases with age, and (2) the mean frequency of the alpha rhythm of the electroencephalogram decreases with age. We hypothesize that the reaction-time differences between age groups can be accounted for, in part, by differences in the alpha frequencies observed between the two groups. We also hope to show that the frequently reported variability in simple reaction time, observed in the same individual over a group of trials, is a function of the alpha frequency present at the time of stimulation.

Method: A simple reaction-time procedure, in which the subject responds to a dim light which is extinguished at intervals when the alpha rhythm is present, is used. The subject responds to the disappearance of the light by uttering a sound. Alpha waves in the period of time between stimulus and response are counted and the frequency of the alpha for each stimulus-response pair is determined. The same kind of data is also obtained using an auditory tone as the stimulus. Curves of reaction time vs. EEG frequency are plotted for each subject.

b. Principal Investigators: Walter W. Surwillo (2/5 time)
George J. Suci (1/4 time)
Melvin D. Davidoff (1/4 time)

Technical Assistance: Mike Kaplan (full-time summer 1958)
Jesse Yaffa (1/3 time since September)

c. Progress During Past Twelve Months: The project was initiated on June 1, 1958. A reaction-time apparatus which is compatible with EEG recording has been designed and constructed; provision has been made for the study of choice as well as simple reaction time.

Including pilot work, approximately 25 subjects have been tested. In the early stages only visual reaction times were investigated and many subjects did not yield sufficient alpha for plotting a reliable curve. However, since auditory reaction time has been added, considerably more data has become available. The data is encouraging. Five of the subjects studied on visual reaction time show what appears to be a linear relation between reaction time and frequency of the alpha rhythm. The older subjects studied appear to have a slower alpha rhythm than the younger subjects. Similar results are being obtained with the auditory reaction-time procedure. The latter data, however, reveal that another factor in addition to alpha frequency is related to or influencing reaction time; plots of reaction time vs. alpha frequency appear to yield not a single curve but several parallel curves. We suspect this to be the effect of Central Motive State of the subject, i.e., when CMS is high, one curve will be observed relating reaction time and alpha frequency; when, on the other hand, CMS is low, a curve of the same slope will be observed but with a different intercept with the reaction-time axis. Attempts at the manipulation of CMS by the use of instructions has been tried. The results tend to substantiate this thesis. We are presently concerned with adding simultaneous measures of CMS to the testing procedure (see Project G-2-NHI-274).

d. Direction of Current Research: Testing will continue, soon we hope, with some measures of CMS. In conjunction with this project, we are planning three related studies to determine:

1. The relation between alpha frequency and stimulus information in a choice reaction-time task.

2. Whether simple reaction time can be manipulated experimentally by experimentally varying alpha rhythm frequency. We plan to try photic driving of alpha during the auditory task. Interest in a collaborative project using drugs to manipulate alpha frequency has been expressed by a physician on the Baltimore City Hospitals and Johns Hopkins staff.

3. The site or location of the generator or generators producing the alpha rhythm.

2. Patient Days: Not applicable

3. Collaborator: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Walter W. Surwillo
October 30, 1958

GERONTOLOGY BRANCH

Project Report

January 1958 - December 1958

1. a. Title: The function of metal ions in enzymatic reactions.

   b. Principal Investigator: Gunther L. Eichhorn (1/2 time)

   Technical Assistance: Albert Osbahr (1/2 time)

Objectives: To arrive at an understanding of the mechanism by which metal ions participate in enzymatic reactions, and also to determine why different metal ions are required for different enzymatic processes.

Methods Employed: It is assumed that in any metal-catalyzed enzymatic process the metal ion must serve either (1) as an active site, by direct attachment to the substrate, or (2) it must react with the enzyme protein, and thus alter the secondary and tertiary structure of the latter, producing the active configuration. It is postulated that, whenever scheme (1) applies, the metal should be capable of bringing about a non-enzymatic reaction; failure of the non-enzymatic reaction, inversely, would favor scheme (2).

Specially selected enzymatic substrates are treated with metal ions under varying conditions, and the nature of the metal-substrate interactions detected by physical measurements, e.g., spectrophotometry. The interaction of the metals with the enzyme protein is studied by macromolecular techniques, such as ultracentrifugation.

Major Findings: The enolase and aconitase reactions have been selected as examples of enzymatic reactions that accomplish very similar molecular transformations, but require different metal ions, i.e., magnesium and iron (II), respectively. The substrates of both reactions have been found to produce metal complexes of varying stabilities, but evidence for a non-enzymatic reaction has been obtained only for the interaction of iron with the aconitase substrates.

Significance of the Program to the Institute: There is evidence that the aging process is correlated with changes in the activities of cellular enzymes. Studies of enzymatic mechanisms are, therefore, of fundamental interest in the program of the Gerontology Branch.

Direction of Current Research: The kinetics of the reaction of iron (II) with citrate, aconitate, and isocitrate will be studied, and the rates compared with the rates that have been observed for the aconitase reaction.

The interaction of enolase with a variety of metals will be investigated, to determine if magnesium ion behaves differently from other ions. Similar studies will be carried out on the arginase and aspartase reactions, and an attempt will be made to correlate the results obtained from all of these experiments with those reported in the literature, and to arrive at an explanation of metal specificity in enzymatic reactions.

Publications and Awards: None

Prepared by Gunther L. Eichhorn
October 31, 1958

Form No. ORP-2
Oct. 1957

PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
Laboratory of Cardiovascular Physiology

Estimated Obligations for FY 1959

Total:	$217,608
Direct:	$165,000
Reimbursements:	$ 52,608

Serial No. NHI-100

1. Laboratory of Cardiovascular Physiology
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Evaluation of the Guyton A-V Oxygen Difference Analyser.

Principal Investigators: J. Mitchell, M.D.
Mrs. Zena McCallum

Other Investigators: None Technicians: J. Cox
C. Scott

Cooperating Units: None

Man Years (calendar year 1958): Patient Days: None

Total:	1/12 of man year
Professional:	1/12 of man year
Other:	1/12 of man year

Project Description:

Objectives and Major Findings:

Number of experiments: 12
Range covered: 0-22.0 Vol.% O_2 A-V difference.
Analyses: 88 pairs of samples (arterial and venous) were drawn and analyzed in duplicate. Checks to 0.3 Vol.% or better were acceptable by Van Slyke Analyses.

Hematocrits were done on all samples to see if variations in r.b.c. altered the sensitivity of the machine.

Animals: Dogs.
Cannulations:

(1) 9 experiments were done using the superior vena cava and femoral artery.
(2) 1 experiment - coronary sinus and femoral artery.
(3) 2 experiments - both femoral veins and one femoral artery. In these experiments the hind limbs were exercised by electrical stimulation to increase the A-V difference.

Part A. Continued

Results: 72 points below 10 Vol. % A-V difference.
16 points above 10 Vol. % A-V difference.

As can be seen by the graph, the results look very promising in the range 0-10 Vol.% O_2 difference. However, there is considerable scatter in the range 10-22 Vol.% O_2 and so it is obvious that more studies will have to be made in this range to ascertain the reason for this.

Proposed course of project. The apparatus is now being used in the lower ranges on experiments involving cardiac metabolism estimations and the changes occurring in A-V uptake during rest and exercise.

Part B included: No

Serial No. NHI-101

1. Laboratory of Cardiovascular Physiology
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Fluid and Electrolyte Transport Across the Large Intestine

Principal Investigators: I. L. Cooperstein
S. K. Brockman

Other Investigators: None

Cooperating Units: Laboratory of Kidney and Electrolyte Metabolism

Man Years (calendar year 1958): Patient Days: None

Total:	½ of man year
Professional:	½ of man year
Other:	0

Project Description:

Objectives and Major Findings: The following work was concerned with fluid and electrolyte transport across the large intestine, and was done by the use of physico-chemical techniques. This work was presented in part at the American Federation for Clinical Research, Atlantic City, May 1958. This work was divided into the following three parts:

1. The electrical potential generated by the large intestine: its relation to electrolyte and water transfer. This report has been accepted for publication by the Journal of Clinical Investigation. The following is a summary of the pertinent findings.

a) An electrical potential difference (P.D.) of 10-40 mV across the wall of the colon. The lumen negative to the blood.

b) Two active transport mechanisms

i) Sodium absorption
ii) HCO_3 secretion

Part A. (continued)

c) Net H_2O flow depended on b)i, and b)ii.

d) Passive chloride absorption.

e) K^+ transport with the electrical and against the chemical gradient.

II. The effect of strophanthidin and evidence for active K^+ transport, to be submitted for publication.

a) Strophanthidin caused:

i) reduction in the P.D.
ii) reduction in the Na^+ absorption and
iv) the HCO_3^- secretion mechanisms.

b) Potassium

i) active transport when the impermeable ions choline and $SO_4^=$ were placed in the colonic lumen. Potassium moved against the electrical, chemical, and mechanical (hydrostatic pressure or "solvent drag") forces.

III. Rhythmical variations in the P.D. These were associated with intra-colonic pressure changes, and were noted in the stomach and ileum.

Proposed Course of Project: Project completed.

Part B included: Yes

Serial No. NHI-101

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

I. L. Cooperstein and S. K. Brockman: The Electrical Potential Difference Generated by the Large Intestine: Its Relation to Electrolyte and Water Transfer. Journal of Clinical Investigation, in press.

Serial No. NHI-102

1. Laboratory of Cardiovascular Physiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A Study of Oxygen Consumption of the Myocardium During Isometric vs. Isotonic Contraction in Isolated Papillary Muscle.

Principal Investigators:

Saul Winegrad, M.D.

Other Investigators: None Technicians: None

Cooperating Units: None

Man Years (calendar year 1958): Patient Days: None

Total:	1 man year
Professional:	1 man year
Other:	None

Project Description:

Objectives and Major Findings. This project was initiated at the beginning of this year in an effort to study in a simple system oxygen consumption as related to type of contraction in an isolated mammalian papillary muscle. The impetus for this study grew from the previous observations in the whole heart that, for a given amount of external work produced, contractions developing larger tensions with lesser shortening were accompanied by a larger consumption of oxygen than those developing lesser tension with greater shortening. The problems to be overcome in a study using isolated papillary muscle revolved around three main factors.

Firstly, a transducer system had to be devised to be able to control both the tension produced and the degree of shortening. The principle of a lever system with a moving fulcrum is being employed to permit this control. It was initially thought that a small tension strain gauge attached to the end of the lever would be adequate but its limited sensitivity eliminated this possibility. The Instrument Shop feels confident that a lever system attached to the most sensitive standard Statham strain gauge will solve this problem. This is at

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A. (Continued)

present under construction and near completion.

Secondly, a means of measuring relatively small changes in oxygen tension is necessary. Originally, a modified Warburg volumetric method was tried but this included the disadvantages of a continuously changing composition of the medium around the muscle and the difficulty of buffer systems to maintain a constant pCO_2. Polarography was, therefore, employed, and after a series of trials it was found that a Clark type of platinum electrode with a membrane covering was superior to a bare platinum wire. The latter had a 10% decay in current production per hour. A constant perfusion system was devised to maintain the constancy of the muscle environment. Even the Clark has some decay, so a means of by-passing the muscle to give an intra-experimental calibration at any time was added to the system. The difficulty with the Clark electrode is that it must be fitted into an essentially microsystem in a fashion that does not significantly increase the volume of system and so produce substantial lag periods following changes in O_2 tension.

Thirdly, a muscle preparation which is not hypoxic in its center has to be used. There is significant evidence in the literature to suggest that the standard cat papillary muscle preparation is in a state of hypoxia and becomes frankly anoxic at rates of contraction over 50% of the normal resting rate of the cat. High metabolic rate and thickness of tissue are the probable explanation. To overcome this difficulty smaller mammalian papillary muscles at temperatures below 37.4°C were tried, and it was found that a rabbit right ventricular papillary muscle at 33°C could beat at a rate equivalent to the resting one at that temperature for 9-10 hours with only 25% deterioration in performance. This is the tissue now being employed.

Proposed course of project:

The solution to the major problems of technique appears to be almost complete, and when this has been accomplished experiments to evaluate the above described oxygen consumption-type of contraction relationship will be performed.

Part B included: No

Serial No. NHT-103

1. Laboratory of Cardiovascular Physiology
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Measurement of Ventricular Muscle Fiber Length

Principal Investigators: J. Mitchell, R. J. Linden.

Other Investigators: None Technicians: None

Cooperating Units: None

Man Years (calendar year 1958): Patient Days: None

Total: ¼ of man year
Professional: ¼ of man year

Project Description:

The Cushny lever principle has been so modified as to render it susceptible of measuring changes in ventricular muscle fiber length. The instrument consists of two arms, one of which pivots on the shaft of a low inertia microtorque potentiometer. The output of this potentiometer is continuously recorded through a Sanborn D. C. coupling amplifier. The arms of the lever are sewn into two points on the left ventricle and the changes in distance between these two points thereby followed. It is assumed that changes in this distance represent changes in fiber length. To date, the lever has been used in nine dogs.

Proposed course of project: The principal interest in this measurement is to be able to follow changes in diastolic fiber length, systolic fiber length, and rates of change in contraction and relaxation. With the other simultaneously recorded parameters it is hoped to be able to determine changes, if any, in end-diastolic distensibility under the influence of both cardiac sympathetic and vagal stimulation, whether any such changes are due either to the specific influence of the nerve stimulation or changes in the duration of diastole or both and eventually to examine more in detail the supposed relationship between end-diastolic fiber length and myocardial O_2 consumption.

Part B included: No

Serial No. NHI-104
1. Laboratory of Cardiovascular Physiology
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Cardiovascular Response to Exercise.

Principal Investigators: R. J. Linden and J. Mitchell

Other Investigators: None

Technicians: Z. McCallum
E. Purcell
F. Perry
J. Cox

Cooperating Units: None

Man Years (calendar year 1958): Patient Days: None

Total:	¼ of man year
Professional:	¼ of man year
Other:	¼ of man year

Project Description:

Objectives and major findings. This project is in its initial stages and is largely concerned with the development of adequate techniques of study. Three ways of producing exercise in the anesthetized dog have been developed. These are: a) the application of eight ECG electrodes to the thighs and abdomen with a large indifferent electrode under the back, b) a rectal electrode with the indifferent electrode under the back or over the epigastrium (modified from an old French technique of electrical artificial respiration used in 1865), and c) a subarachnoid electrode or electrodes placed at about L 3 or L 4. The application of a rhythmically rising and declining voltage, administered by means of an electrophrenic respirator, produces vigorous contractions which have resulted in O_2 consumptions ten times that observed in the control period. The Guyton recorder has been used for the continuous registration of the arteriovenous O_2 difference between femoral and pulmonary arterial blood. Together with means, not as yet worked out, for the continuous recording of O_2 consumption, continuous (calculated) cardiac output measurements will be made.

Project Description (Continued)

Proposed course of project. Together with the left atrial pressure (bronchoscope) and right atrial pressure, as well as intrapleural and pulmonary and aortic pressures, it is anticipated that data for the construction of ventricular function curves can be obtained. It is the object of this study to ascertain what effect, if any, exercise has on the ventricular function curve and also the importance, if any, of autonomic nerves and the baroreceptors in producing any observed curve shift.

Part B included: No

Serial No. NHI-105
1. Laboratory of Cardiovascular Physiology
2:
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Neuronal Control of Ventricular Function: Influence of Autonomic Efferent Fiber and Carotid Sinus Stimulation on the Relationship Between Filling Pressure and Stroke Work.

Principal Investigators: S. J. Sarnoff, S. K. Brockman, and J. P. Gilmore

Other Investigators: R. J. Linden, J. Mitchell

Technicians: E. Purcell
F. Perry
C. Whitted

Cooperating Units: None

Man Years (calendar year 1958): Patient Days: None

Total:	1 man year
Professional:	1 man year
Other:	1 man year

Project Description:

Objectives and Major Findings. Measurements were made of atrial, arterial and ventricular pressures while metering cardiac output. Pressure and flow were also measured in the independently perfused carotid arteries with exclusion of other arterial supply to the head. Heart rate was held constant in all experiments by atrial stimulation. Observations were made which demonstrated that stimulation of the isolated left stellate ganglion shifted the ventricular function curve to the left and that the extent of the curve shift is a function of the frequency and/or voltage of the applied stimulus. Stimulation of the distal cut end of either vagus produced the opposite effect, that is, a curve shift to the right. The order of magnitude of the changes observed was large. These experiments make it possible to put Starling's Law of the Heart in clearer perspective. That is, the central nervous

Part A, continued.

system has available to it efferent pathways by means of which it can systematically manipulate the relationship between filling pressure and ventricular stroke work.

Further experiments were then done to ascertain whether these efferent pathways are exercized by changes in carotid sinus pressure. Stimulation of the carotid sinus nerves produced hemodynamic responses identical with those observed when withdrawing sympathetic stimulation or initiating vagal stimulation, that is, a shift of the ventricular function curve to the right. Elevation of the carotid sinus pressure had the same effect. Lowering the carotid pressure shifted the ventricular function curve to the left. The observed changes were marked. In summary, these data demonstrated that the organism has available to it pathways by means of which the heart is caused to contract more forcefully at any given filling pressure when arterial pressure is low and, conversely is caused to contract less forcefully at any given filling pressure when arterial pressure is high.

It was further observed that, with large changes in carotid pressure, total peripheral resistance changed one to two fold while changes in ventricular external work increased more than tenfold at the same or even lower filling pressures. These data suggest that the dominant consequence of baroreceptor stimulation for circulatory regulation is the reflex effect on myocardial contractility rather than on peripheral vascular tone.

Proposed course of project. This project per se is completed but the implications of the conclusions arrived at will form the basis for the design of further investigation (for example, see accompanying project entitled "The Cardiovascular Response to Exercise.").

Serial No. NHI-100

1. Laboratory of Cardiovascular Physiology
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Effect of Stellate Stimulation on Arterial Pressure, Atrial Pressure and Renal Function.

Principal Investigator: J. P. Gilmore

Other Investigator: S. J. Sarnoff Technicians: William D. Fisher

Cooperating Units: None

Man Years (calendar year 1958): Patient Days: None
Total: One-third of man year.
Professional: One-third of man year.
Other: One-third of man year.

Project Description:

Objectives: (Methods employed). Stimulation of the isolated stellate ganglion in the dog.

Major findings: Preliminary experiments to date demonstrate:

1) Upon stimulation of the isolated stellate ganglion in the dog there occurs an immediate diuresis which appears to be independent of arterial pressure.

2) During the diuresis glomular filtration rate usually shows little change and total urinary solute excretion increases.

3) Following vagotomy, the diuresis is significantly reduced although the elevation of arterial pressure is at least the same or, more frequently, greater than that obtained before vagotomy.

4) Left atrial pressure decreases during stellate stimulation both prior to and following vagotomy.

Part A. (Continued)

5) The data suggest that, although there may be receptors in the heart which upon adequate stimulation can modify urine flow as suggested by Gauer and co-workers, the adequate stimulus does not appear to be an increased left atrial pressure.

Proposed course of project: To continue the above experiments in an attempt to ascertain the mechanisms involved in "stellate diuresis."

Part B included: No

Serial No. NHT-107

1. Laboratory of Cardiovascular Physiology
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Relationship between Osmotic Pressure and Caliber of Blood Vessels.

Principal Investigator: S. I. Yamada

Other Investigator: None Technicians: Joseph Miles

Cooperating Units: None

Man Years (calendar year 1958): Patient Days: None
Total: ¼ of man year.
Professional: ¼ of man year.
Other: ¼ of man year.

Project Description:

Routine methods were employed.

The major findings are described as follows:

Experiments were recently started to investigate the role played by osmotic pressure in influencing the caliber of blood vessels (as manifested by changes in resistance to blood flow) in different peripheral as well as visceral areas. Particular attention is being paid to the complication of this factor, thus far neglected, in the measurement of the so-called "critical closing pressure" of a vascular bed. Infusion of hypotonic fluid intravenously into the cat decreases the resistance to flow in the kidney, probably mainly because of decreased viscosity, and elevates the critical closing pressure in this organ, probably because of increased extravasation of fluid into the interstitial spaces and, as a result of this, because of increased tissue pressure. Infusion of hypertonic fluid has the opposite effect.

Direction of current research:

To measure *in vitro* the viscosity of the perfusate (blood as well as homogeneous fluids) and to measure *in vivo* the osmotic

Part A (continued).

pressure of the perfusate and relate changes in these factors to changes in resistance to flow and critical closing pressure in the kidney and hind leg of the cat. Construction of a constantly-recording osmometer with the use of a pressure transducer is now being planned.

Part B included: No

Serial No. NHI-108
1. Laboratory of Cardiovascular Physiology
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A Study of a Mesenteric Barosensitive Mechanism and its Role in the Reflex Regulation of Arterial Pressure in the Cat.

Principal Investigators: S. J. Sarnoff and S. I. Yamada

Other Investigators: None Technicians: Joseph Miles
Burton Alter

Cooperating Units: None

Man Years (calendar year 1958): Patient Days: None

Total: 3/4 of man year
Professional: 3/4 of man year
Other: 3/4 of man year for Joseph Miles
¼ of man year for Burton Alter

Project Description:

Intravascular hypotension in the cat abdominal viscera supplied by coeliac, superior mesenteric and inferior mesenteric arteries produced substantial increases in the systemic arterial pressure and heart rate. In the majority of cases, this effect was seen even with intact carotid sinus and aortic arch baroreceptive systems but was accentuated after vagotomy and carotid sinus denervation. Intravascular pancreatic hypotension also produced elevations in the systemic arterial pressure. These pressure rises were usually two thirds to three quarters as great as those observed during occlusion of the three major abdominal vessels. Intravascular hypertension, on the other hand, in the superior mesenteric artery caused a fall in the systemic arterial pressure. From nerve section experiments, it was concluded that the afferent of this abdominal baro-sensitive system were not carried in the vagus nerve, but rather in splanchnic afferent fibers. The reflex effects of this system were blocked by tetraethyl ammonium and hexamethonium chloride.

Part A (continued).

Proposed course of project: It is anticipated that further studies may be initiated to a) define the pathways more precisely by splanchnic nerve section, and spinal cord section at various levels; b) ascertain the presence or absence of these receptors in the dog, monkey and possibly man.

Part B included: Yes

(Attachment I)

Serial No. NHI-108

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Sarnoff, S. J. and Yamada, S. I.: Evidence for Reflex Control of Arterial Pressure from Abdominal Receptors with Special Reference to the Pancreas. Circulation Research In press.

Honors and Awards: None

Serial No. NHT-100
1. Laboratory of Cardiovascular Physiology
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Effect on Myocardial Contractility of Ventricular versus Atrial Excitation

Principal Investigators: S. J. Sarnoff, S. K. Brockman and J. G. Gilmore

Other Investigators: R. J. Linden, J. Mitchell

Technicians: E. Purcell
F. Perry
C. Whitted

Cooperating Units: None

Man Years (calendar year 1958): Patient Days: None

Total:	3/4 of man year
Professional:	3/4 of man year
Other:	3/4 of man year

Project Description:

Objectives and Major Findings: As the result of an incidental observation that filling pressure rose and ventricular work fell when the site of electrical excitation was abruptly changed from atrium to ventricle, a systematic study was initiated to study this phenomenon.

Pressures were measured in left and right atria, pulmonary artery and aorta and, in some experiments, also in the left ventricle. Total aortic flow (C.O. minus coronary flow) was also recorded and by previously developed techniques, data then obtained for the construction of ventricular function curves. It was observed that the change from an atrial to a ventricular site of excitation produced a pronounced shift of the ventricular function curve to the right, that is, less external work at any given filling pressure. In many instances, this effect was such that there was more than 50% decrease in external work at the same filling pressure. It was also observed that myocardial oxygen consumption rose when going from atrial to ventricular stimulation which, since external work fell, resulted in a decrease in myocardial efficiency.

PHS - NIH
Individual Project Report
Calendar Year 1958

One possible explanation for the observed phenomena being entertained is that the contraction of ventricular fibers is less synchronous when one excites what is essentially an ectopic focus than when the impulse is propagated normally. In support of this view are the observations by others that the electrical propagation of an impulse originating in the ventricle is slower than an impulse of atrial origin. We had a further strengthing of this interpretation from our observations that the upslope of ventricular pressure is less steep as is the curve showing the rate of fiber shortening as measured by the newly developed length lever. That is, if the impulse is less well coordinated and propagated less rapidly, the initial and last fibers to contract are doing so with the remainder of the ventricle acting as a flaccid aneurysm more than would be the case when the contraction is more synchronous.

We can only guess at the significance of these findings for clinical heart disease. They would appear to furnish a more realistic basis for explaining the consequences of ventricular arrhythmics than has been previously available, especially when compounded by tachycardia. One is also impelled to wonder whether certain degrees of asynchronous myocardial contraction might contribute to the limited performance characteristics observed in clinical heart disease.

Proposed Course of Project. Further research not immediately contemplated. Publications and manuscripts under preparation.

Part B Included: No

Attachment 49

Serial No. NHI-110
1. Laboratory of Cardiovascular Physiology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Possible Physiological Significance of Vasodilator Substances in Human Urine

Principal Investigators:

Pierce, J. V.
Sarnoff, L. C.
Sarnoff, S. J.
Webster, M. E.

Other Investigators: None Technicians: Fisher, Wm. D.
Wagner, P. A.

Cooperating Units: Laboratory of Chemistry of Natural Products

Man Years (calendar year 1958): Patient Days: None

Total:	1 man year
Professional:	1 man year
Other:	1 man year

Project Description:

Earlier studies have shown that patients with orthostatic hypotension gave a decreased urinary excretion of a non-dialyzable vasodilator substance. Further studies have been conducted to determine the identity and physiological significance of this substance.

It had previously been noted that this non-dialyzable vasodilator had biochemical properties similar to callicrein, a hypotensive enzyme of endogenous origin. Correlation of a series of partially purified preparations from hog and bovine pancreas and from human urine for their vasodilator and hypotensive effect indicated that the biological activities were measuring one substance.

Callicrein is reported to exert its hypotensive effect by enzymatic action on callidinogen, present in normal plasma, to

Part A. Continued

(Project Description)

release a smaller molecule, presumably polypeptide, called callidin. Callidin can be differentiated from callicrein by its ability to contract guinea pig intestine, and the callidinogen content of plasma or serum can be determined by addition of an excess of callicrein to the tissue bath. It was found that certain crude callicrein preparations from hog and bovine pancreas failed to cause contraction of the tissue when added to human plasma, although more highly purified preparations from bovine pancreas were satisfactory in this regard. All callicrein preparations from human urine when added to human plasma furnished maximal contraction of the tissue. Since the addition of human urinary callicrein to plasma, previously treated with crude hog pancreatic callicrein, still fails to release a substance capable of contracting the intestine, it is possible that this hog pancreatic callicrein had digested the callidinogen but contained an enzyme which immediately destroyed the polypeptide. Infusion of relatively large amounts of the hog pancreatic callicrein (300 Frey units) into dogs (2) caused a lowering of the blood pressure and a reduction of their callidinogen content of approximately 60% (sample taken three hours after infusion).

As an initial approach to this biological system of callicrein, callidinogen, callidin and their respective inhibitors, it was decided to attempt the isolation of callidinogen, the substrate for initial enzymatic activity of callicrein. However, heavy losses have been incurred in all purification procedures tried to date. It was found that out-dated citrated plasma was an excellent source for callidinogen. However, Cohn's fractions prepared by two manufacturers gave very poor yields of callidinogen (10%). Callidinogen was stable in citrated plasma for at least one month at pH 6.0. However, at more alkaline pH, the callidinogen content was markedly lowered in three to six days storage at 4°C. This was due to activation of one of the plasma proteinases, since heating the plasma for two hours at 56°C or the addition of crystalline soy bean trypsin inhibitor (SBTI) prevented the destruction of the callidinogen. Dialysis has also been found to activate proteinases which digest callidinogen and are inhibitable with SBTI. Since both plasmin and blood callicrein are known to be capable of digesting callidinogen and both proteolytic activities may be inhibited with SBTI, it is possible that either one or both of these proteinases are activated by destruction or removal

Part A, Continued

(Project Description)

of their inhibitors.

Callicrein can now be prepared from human urine by direct-batch-wise adsorption on XE-64 resin. Callicrein prepared by this method is five times purer than callicrein prepared by adsorption on uranium acetate. Preliminary studies have been initiated on the best method for the initial extraction and precipitation of callicrein from human pancreas.

Current studies being conducted to determine the role of the callicrein-callidinogen-callidin system in the regulation of blood pressure *in vivo* are not sufficiently definitive at this time to report.

Proposed course of project:

As techniques are developed for their measurement, more experiments will be conducted to determine what role the callicrein-callidinogen-callidin system plays in the regulation of blood pressure. This will include patient studies, acute and chronic experiments in animals in which blood pressure will be varied.

Continued studies will be made on the purification of callidinogen from human plasma and the purification of callicrein from human urine and from human pancreas.

Part B included: Yes

(Attachment I)

Serial No. NHI-110

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Sarnoff, S. J., Case, R. B., Macruz, R., Sarnoff, L. C., Sussman, K. E., and Pierce, J. V. Observations on the Vasodilator Properties of Urine. Circulation Res. 6: 522-537, 1958.

Honors and Awards: None

Form No. ORP-2
Oct. 1957

PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
Laboratory of Cellular Physiology and Metabolism
Cellular Physiology Section

Estimated Obligations for FY 1959

Total: $260,472

Direct: $198,000

Reimbursements: $ 62,472

Serial No. NHI-111

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS--NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: The structural role of proline and hydroxyproline in proteins

Principal Investigators: William F. Harrington
Michael Sela
Peter H. von Hippel

Cooperating Units: Naval Medical Research Institute, Bethesda, Md.
(Dr. Peter H. von Hippel)

Man Years (calendar year 1958)
Total: 1/2
Professional 1/2

Patient Days: None

Project Description:

Objectives: To gain information on the relationship between the proline residues of proteins and the specific configuration of polypeptide chains observed in the globular and fibrous proteins.

Methods:

1. The configuration of proline in various simple copolymers of glycine and proline has been investigated mainly by means of optical rotation. These copolymers are model structures for gelatin, and the effects of various solvent systems as well as temperature on the properties of the copolymers have been measured and compared to gelatin.

2. The extent of hydrolysis of various glycine-proline copolymers through the action of collagenase has been studied. The collagenase is known to be highly specific, the only known protein substrate being collagen. Cleavage of polypeptide chains containing only glycine and proline was followed by continous titration procedures as well as by the liberation and identification of the α-amino groups released.

3. The process of gelation and the formation of the collagen-type fold during the gelling process at low temperature has been extensively studied using the enzyme collagenase as a probe of the configurational changes in the gelatin molecule. Kinetics of the hydrolysis of gelatin at various temperatures by collagenase have been followed by comparable studies in the pH stat and in the polarimeter.

Major Findings:

1. Aqueous solutions of neutral salts which have large effects on the configurational properties of poly-l-proline and on the proline residues in glycine-proline copolymers also have profound effects on the properties of collagen and gelatin suggesting that the peptide configuration about proline-proline bonds in these proteins is altered in these solvents.

2. The effect of temperature on the optical rotatory properties of gelatin parallels that observed for the glycine-proline copolymers. Since glycine residues to not exhibit optical rotation, the changes in rotation observed must be related to the configuration of the proline residues about the peptide bonds. It is suggested that at low temperatures the transconfiguration of proline-proline bonds is predominant. At higher temperatures rotation of one proline with respect to its neighbor can occur and this eliminates the structural pattern established at low temperatures.

3. From the work with glycine proline copolymers it appears that the enzyme collagenase requires the specific sequence -- X-glycine-proline -- in a polypeptide chain in order for hydrolysis to take place. Cleavage of the chain occurs between the nonspecific amino acid, X, and glycine. This information may be of value in future studies on other as yet uninvestigated proteins in which this particular sequence may occur. For example, the anterior pituitary hormone β-MSH is known from structure work to possess this sequence.

4. An examination of the hydrolysis of gelatin by collagenase reveals that at temperatures below 25° the normally random polypeptide chain of gelatin folds into a much more organized structure. The special configuration of the gelatin chain below 25° apparently is closely related to the gelation process and to the formation of the unique structural pattern associated with collagen. It is believed that the structural changes at low temperature results from Cis-trans isomerizations at the proline-proline or proline-hydroxyproline peptide bonds in the gelatin chain.

Significance to Heart Research: This work should help in clarifying the properties of certain proteins containing unusually high amounts of proline and hydroxyproline. Collagen and casein are examples of proteins whose structural properties appear to be primarily related to the proline residues. Collagen, of course, is the most important structural protein in the body.

Serial No. NHI-111

Proposed Course of Project:

1. Studies on collagens and gelatins from various sources with differing proline and hydroxyproline content will be continued.

2. Similar studies on α and β casein will be initiated. These proteins have very high proline contents and preliminary investigation has shown that certain of their properties are closely similar to those observed for gelatin.

3. Examination of simple model systems containing proline and hydroxyproline will be continued in collaboration with members of the Weizmann Institute, Israel.

PART B included Yes X No

Serial No. NHI-111

PHS--NIH
Individual Project Report
Calendar Year 1958

PART B: Honors, Awards, and Publications

Publications other than obstracts from this project:

W. F. Harrington and Michael Sela, Studies on the Structure of Poly-l-Proline in Solution, Biochim. et Biophys. Acta, 27, 24-41 (1958).

W. F. Harrington, Effect of Neutral Salts on the Structure of Collagen and Gelatin, Nature, 181, 997-998 (1958).

Serial No. NHI-112

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Md.

PHS--NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Use of proteolytic enzymes as probes of the secondary structure of fibrous proteins

Principal Investigators: William F. Harrington
Peter H. von Hippel
Parker Small
Walter Englander

Cooperating Units: Naval Medical Research Institute, Bethesda, Md. (Dr. Peter H. von Hippel)

Man Years (calendar year 1958) Patient Days: None
Total: .9
Professional: .9

Project Description:

Objectives: It is well known that denatured or unfolded protein molecules are attacked much more readily by proteolytic enzymes than are their native counterparts. Recent developments suggest that many native protein molecules may be only partially folded and it seems likely, therefore, that the polypeptide chains in the amorphous regions of these protein molecules may be more rapidly cleaved than are the crystalline areas. Careful analysis of the kinetics of proteolysis should be of considerable value in elucidating the fine structure of these protein macromolecules.

Methods: Two fibrous proteins have been examined in detail, viz., myosin and collagen. The kinetics of proteolysis by trypsin and collagenase respectively have been followed by several physical and chemical methods including continuous titration (pH stat), appearance of nonprotein nitrogen, optical rotation and viscosity.

Major Findings:

1. About 30 per cent of the ichthyocol collagen molecule is less firmly folded than the remaining 70 per cent. The "amorphous" regions of collagen have a much more restricted configuration than the gelatin molecule.

Serial No. NHI-112

2. About 20 per cent of the myosin molecule appears to be "amorphous" or possesses a much looser hydrogen-bonded fabric than the remaining 80 per cent. The "amorphous" areas of myosin appear to occur at about 1/4 and 1/2 the length of the myosin rod as judged by the size of the high molecular weight degradation products which are rapidly formed on enzymatic attack.

Significance to Heart Research: This work is aimed at a better understanding of the aspects of structure which make a protein fibrous in character. Fibrous proteins, including fibrinogen, the collagens and the muscle proteins, appear from the present work to share many similar features and the systematic study of these proteins along the lines described above should help to unearth any common structural patterns.

Proposed Course of Project:

1. Studies of the secondary structure of fibrous proteins utilizing proteolytic enzymes will be extended to other fibrous proteins such as fibrinogen.

2. Model polypeptide chains such as oxidized ribonuclease and simple synthetic copolymers will be examined.

Part B included Yes X No

Serial No. NHI-112

PHS--NIH
Individual Project Report
Calendar Year 1958

PART B: Honors, Awards, and Publications

Publications other than abstracts from this project:

W. F. Harrington, P. H. von Hippel, and E. Mihalyi, Proteolytic Enzymes as Probes of the Secondary Structure of Fibrous Proteins, (in press).

Serial No. NHI-113
1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH
Individual Project Report
Calendar Year 1958

Part A. Project Description

Project Title: Energy transfer associated with electron transport

Principal Investigators: W. Wayne Kielley
J. Ramsey Bronk

Other Investigators: Lisa Barnett (technical)
Clarence Edwards (summer) (technical)

Man Years (Calendar year 1958): Patient Days: None
Total: 1 3/4
Professional: 1
Technical: 3/4

Project Description:

Objectives:

In most aerobic cells the major pathway for disposal of electrons arising from oxidative reactions leads to oxygen through a system of closely integrated protein and lipid elements forming the membranes of the mitochondrial components of the cell. Integrated into this lipoprotein system is a mechanism for transferring the energy available in the sequence of electron transfers into the form of "high energy" phosphate bonds, providing a major source of energy for aerobic organisms.

In this lipoprotein system the sequence of electron transfers is incompletely known, few of the individual elements have been separated and characterized, the nature of the intermediate in energy transfer is unknown and we can only speculate as to the function of the phospholipid components. These problems and the questions of physiological control mechanisms form the broad objectives of this program

Progress in understanding the mechanism of the oxidative phosphorylation has been greatly facilitated by the development in this and other laboratories of procedures for fragmenting mitochondria to yield much smaller units capable of carrying out the integrated oxidative phosphorylation reactions free of interfering and complicating reactions present in intact mitochondria. Previous reports have dealt with the properties of these submitochondrial particles, and association of two exchange reactions with the phosphorylation process, the development of a reaction scheme and point of action of dinitrophenol in

dissociating oxidation and phosphorylation. During the past year efforts have been directed toward further investigation of the exchange reactions and their involvement in the phosphorylation process. The influence of thyroxin on the system has also been extensively investigated.

Methods Employed:

1. A study of the effects of specific sulfhydryl-binding agents on electron transport, phosphorylation and the exchange reactions indicates that an SH group is involved in the transfer of energy prior to participation of phosphate and is apparently the site of action of dinitrophenol.

2. It has been known for a long while that thyroxin will "uncouple" oxidation and phosphorylation in intact mitochondria, and this effect has been proposed as a basis for the physiological action of thyroxin. However, such effects occur only at high thyroxin concentrations and after an extended preincubation. The effect of thyroxin and related compounds on the submitochondrial system have been extensively investigated in this laboratory. An immediate effect of thyroxin and analogues with thyroxin activity is an acceleration of oxidation without altering the phosphorylative efficiency. At higher concentrations and following preincubation, uncoupling can be observed. The observations suggest that the latter effect is secondary, but it is still uncertain whether the acceleration of oxidation can be regarded as physiologically significant.

Significance to Heart Research: Of the energy made available to cells through oxidation of sugars about 75-80% arises through the process of electron transport phosphorylation. The mechanism of this latter process is still unknown. Its importance in the energy relationships of all aerobic cells -- including heart tissue -- is evident.

Proposed Course of Project: Present efforts are directed along two lines:

1. Separation of the ADP-ATP exchange reaction from the rest of the system, an examination of its properties in the isolated state and eventual attempts to reconstruct other parts of the reaction sequence.

2. Considerable evidence has accumulated that quinones identical with or related to vitamins K and E may be involved in electron transport and its associated phosphorylation. We have examined the behavior of the submitochondrial system with a number of compounds of this type. Some have exhibited a marked acceleration of phosphorylation and these effects are currently under investigation.

Part B included: Yes X No

PHS--NIH
Individual Project Report
Calendar Year 1958

Serial No. NHI-113

Part B: Honors, Awards, and Publications

Publications: (other than abstracts from this project)

W. W. Kielley and J. R. Bronk, Oxidative Phosphorylation and Associated Reactions in Mitochondrial Fragments Obtained by Sonic Vibration. In Proceedings of the International Symposium on Enzyme Chemistry, Tokyo and Kyoto, 1957, Academic Press, New York, 1958.

J. R. Bronk, Some Effects of Thyroxin on Oxidative Phosphorylation in Submitochondrial Particles and Intact Mitochondria, Biochim. Biophys Acta, 27, 667-668 (1958).

J. R. Bronk and W. W. Kielley, Evidence for the Point of Action of 2,4-Dinitrophenol on ATPase, ATP-^{32}P Exchange and Phosphorylation, Biochim. Biophys. Acta, 29, 369-377 (1958).

W. W. Kielley and J. R. Bronk, Oxidative Phosphorylation in Mitochondrial Fragments Obtained by Sonic Vibration, J. Biol. Chem., 230, 521-533 (1958).

Serial No. NHI-114
1. Laboratory of Cellular Physiology
2.
3. Bethesda, Maryland

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH
Individual Project Report
Calendar Year 1958

Part A. Project Description

Project Title: Biochemistry of muscular contraction

Principal Investigator: W. Wayne Kielley

Collaborators: William F. Harrington
William J. Dreyer

Other Investigator: Lisa Barnett (technical)

Man Years (Calendar year 1958) Patient Days: None
Total: 1-1/4
Professional: 3/4
Technical: 1/2

Project Description:

Objectives: It was observed some time ago that the ATPase activity of myosin, the protein unit of the contractile mechanism, exhibits a biphasic response to titration of the SH groups, with a 3-4 fold stimulation of ATPase activity occurring when approximately 1/2 of the SH groups are titrated, further titration leading eventually to complete inhibition. The question exists whether the two phases of this response are due to titration of specific groups or to statistical titration of the groups involved in each active center. It was planned to approach the problem using differential labeling of the groups with radioactive SH reagents followed by tryptic digestion and application of the "fingerprint" technique to identify the cysteine containing peptides. However, interpretation of the results would depend on whether the large myosin A molecule consists of a single or several peptide chains and if several, whether they are identical. Therefore, it was decided to approach this question first, using concentrated guanidine hydrochloride to disrupt all of the secondary and tertiary structure, leaving only the primary covalent bonds of the peptide chain. Examination in the ultra centrifuge should then establish the sizes of the basic units. Myosin A is a rather large molecule (400,000 molecular weight) but offers a unique opportunity to establish some details of fine structure. It is a very asymmetric molecule (1600 Angstrom units long and 21 Å in diameter) and the number of ways of arranging a single or several peptide chains in this shape are very restricted.

Serial No NHI-114
1. Laboratory of Cellular Physiology
2.
3. Bethesda, Maryland

PUBLIC HEALTH SERVICE -- NATIONAL INSTITUTES OF HEALTH
Individual Project Report
Calendar Year 1958

Part A. Project Description

Project Title: Biochemistry of muscular contraction

Principal Investigator: W. Wayne Kielley

Collaborators: William F. Harrington
William J. Dreyer

Other Investigator: Lisa Barnett (technical)

Man Years (Calendar year 1958) Patient Days: None
Total: 1-1/4
Professional: 3/4
Technical: 1/2

Project Description:

Objectives: It was observed some time ago that the ATPase activity of myosin, the protein unit of the contractile mechanism, exhibits a biphasic response to titration of the SH groups, with a 3-4 fold stimulation of ATPase activity occurring when approximately 1/2 of the SH groups are titrated, further titration leading eventually to complete inhibition. The question exists whether the two phases of this response are due to titration of specific groups or to statistical titration of the groups involved in each active center. It was planned to approach the problem using differential labeling of the groups with radioactive SH reagents followed by tryptic digestion and application of the "fingerprint" technique to identify the cysteine containing peptides. However, interpretation of the results would depend on whether the large myosin A molecule consists of a single or several peptide chains and if several, whether they are identical Therefore, it was decided to approach this question first, using concentrated guanidine hydrochloride to disrupt all of the secondary and tertiary structure, leaving only the primary covalent bonds of the peptide chain. Examination in the ultra centrifuge should then establish the sizes of the basic units. Myosin A is a rather large molecule (400,000 molecular weight) but offers a unique opportunity to establish some details of fine structure. It is a very asymmetric molecule (1600 Ångstrom units long and 21 Å in diameter) and the number of ways of arranging a single or several peptide chains in this shape are very restricted.

Serial No. NHI-114

Methods Employed: All observations so far are of a preliminary nature. Much time has been spent in solving technical problems, particularly in the ultra centrifuge experiments where there is little recorded experience in the use of concentrated salt solutions particularly in the approach to equilibrium (Archibald) technique of determining molecular weights. We, therefore, engaged in a long series of experiments using ribonuclease as a very well characterized model for determining the influence of concentrated guanidine hydrochloride solutions on centrifugation of protein. At present, we can say that the myosin A molecule is made up of three or four peptide chains, all identical in weight. Typtic digestion and fingerprinting of peptides also indicates a unit weight of about 100,000 and, in addition, also indicates that the 3 or 4 major chains are all identical. There is a suggestion of a small amount of smaller components, but we are unable to say whether they are part of the myosin A or are present as contamination.

Significance to Heart Research: There is no immediate significance to heart research, though more extensive knowledge of the proteins involved in muscular contraction may be of eventual importance.

Proposed Course of Project: The assignment of reliable numbers to the weight of the subunits of myosin requires an extensive analysis and these experiments are continuing. Only preliminary fingerprint experiments have been performed so far and technical questions still exist. An additional problem under investigation, using this technique, is the identity or non-identity of myosin and some of the other proteins implicated in the structure of the myofibril. No end groups have so far been reported for the myosin molecule, and we are currently attempting to do this. An answer to the initial question of the SH groups has so far not been attempted.

Part B included: Yes No X

Serial No. [illegible]-115
1. Laboratory of Cellular [illegible]
2.
3. Bethesda, Maryland

PHS--NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: On the nature of the self-absorption phenomenon for Carbon-14.

Principal Investigator: Richard W. Hendler

Other Investigator: Jay Yedvab (technical)

Man Years (Calendar year 1958) Patient Days: None
Total: 3/10
Professional: 1/5
Technical: 1/10

Project Description:

Objectives and Methods Employed: Carbon-14 is the most widely used isotope in biological research. Because of the low energy of its emission, a substantial fraction of the radiation contained is absorbed by the sample itself and hence escapes detection. It is essential to be able to correct for the amount of radiation lost in the sample. Past theoretical treatment assumed that this loss of detectable radiation followed an exponential decrease with increasing weight of sample. It has been observed in this laboratory that it is not exponential but hyperbolic. The practical significance of this finding is that by plotting a reciprocal function, a straight line graph is obtained for a correction factor vs weight of sample per unit area. This factor is highly accurate over the entire range of weights encountered. It is very easy to obtain and has been found to apply to many different counting situations involving different type counting equipment and radioactive materials. The exponential treatment has been found to lead to significant errors. Aside from the obvious practical advantages in the ease of application and greater accuracy of the new treatment, certain theoretical considerations are involved. The exponential treatment is based on the assumption that each layer of an absorbing substance will always absorb a constant per cent of the impinging radiation. The hyperbolic treatment, on the other hand, is a consequence of the variability of the ability of each layer to absorb radiation. An expression has been derived which describes the variability as a function of weight of sample and energy spectrum of the radioactive emission.

It has been found that the same situation seems to apply for Sulfur-35, and the possibility exists that all weak wide spectrum beta emitters may fall into the same category.

Serial No. NHI-115

Significance to Heart Research: Much of heart research today is carried out by use of isotopes, particularly C^{14}, and this method enables necessarily rapid and accurate correction for counting data.

Proposed Course of Project: It is not known at present whether this investigator will have time to further pursue this project.

Part B included: Yes No X

Serial No. NHI-116

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS--NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: A study of the mechanism of protein biosynthesis in the hen oviduct

Principal Investigator: Richard W. Hendler

Man Years (calendar year 1958)
Total: 4/5
Professional: 4/5

Patient Days: None

Project Description:

Objectives: The mechanism of protein biosynthesis is one of the most important areas in biochemistry about which the least is known. It is the purpose of this work to study the basic reactions of protein biosynthesis in a tissue highly specialized for performing this function, the oviduct of the laying hen.

Methods and Major Findings: The existence of an intermediate stage between free amino acid and protein was indicated by the following observation. Free amino acid does not become incorporated into protein in a disrupted cell preparation whereas, if the cells are labeled prior to disruption, radioactivity can enter the protein from some labeled precursor in the cells. An attempt was made to locate the source of this radioactivity as well as to examine other cellular constituents for a possible precursor role. The entire tissue was examined in different chemical fractions, for the rate of accumulation radioactive amino acids, the absolute level of accumulation, and the dynamic state of the radioactivity in relation to the rate of incorporation of the amino acids into protein. Preliminary steps were taken in characterizing the chemical nature of the binding of amino acids in these "non-protein" fractions. The results indicate that, contrary to expectations, the nucleic acid fraction does not behave as would be expected for a role of intermediate carrier of amino acids. The lipid fraction, on the other hand, demonstrated kinetic behavior with reference to amino acid uptake and turnover, entirely consistent with the possibility of its playing the role of intermediate carrier for the amino acids. This idea was further strengthened by the observation that agents stimulatory to lipid synthesis stimulated amino acid incorporation and agents detrimental to lipid structure strongly inhibited amino acid incorporation. The possibility of lipids being involved in protein synthesis has hitherto been ignored. However, on cytological and thermodynamic grounds the possibility could offer distinct advantages.

Serial No. NHI-116

Significance to Heart Research: The dry weight of the body and its soft tissue structural material is mainly protein. The Heart Institute is concerned with understanding the normal function of heart tissue. The heart as a muscle is in a constant state of protein synthesis and degradation. Biochemical knowledge is based on the thesis that basic biochemical mechanisms are similar in the varied types of living tissue. By studying a tissue highly specialized with respect to the process of protein synthesis (oviduct of the laying hen) it is hoped that knowledge of this vital phenomenon will be applicable to the tissues of man.

Proposed Course of Project: The isolation, purification, and characterization of the lipid-amino acid complex will be pursued. The possible role of these compounds in protein biosynthesis will be further studied.

Part B included Yes X No

Serial No. NHI-116

PHS--NIH
Individual Project Report
Calendar Year 1958

PART B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Richard W. Hendler, Possible Involvement of Lipids in Protein Synthesis, Science, 128, 143-144 (1958).

Richard W. Hendler, Passage of Radioactivity between Protein Fractions of a Hen Oviduct Homogenate, J. Biol. Chem., 229, 553-561 (1957).

Serial No. NHI-117

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS--NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Studies of the mechanism of genetic control of protein synthesis

Principal Investigators: William J. Dreyer
Arnold M. Katz
Christian B. Anfinsen

Cooperating Units: Gebhard Koch, Cold Spring Harbor, Long Island (2 months on part 3 (Methods))

Other Investigators: Judith Wegman (technical) (June-August)
Jack Blount (technical) (Jan.- March)

Man Years: (calendar year 1958) Patient Days: None
Total: 2 years
Professional: 1 1/2
Technical: 1/2

Project Description:

Objectives: Recent genetic studies have made it clear that the genetic material, deoxyribonucleic acids (DNA), of living organisms exerts rigid control over the synthesis of proteins. It appears that a change in the chemical structure of DNA due to a mutation can result in a change in the structure of a protein synthesized under the control of the altered "gene". The structural analysis of such altered proteins should provide useful data relating to the translation of genetic information into protein structure.

The genetic systems and the proteins currently being studied are those of the very simple "organisms", bacterial viruses.

Methods:

1. Fractionation of bacterial virus proteins:

The proteins of the T2 bacterial virus have been separated into several components by means of column chromatography in the presence of high concentrations of urea. Urea has been chosen because it dissociates the virus proteins, permits their fractionation on ion exchange columns, and does not irreversibly destroy their serological activity. Using a serological assay, the serum blocking antigen has

been located in one of the several protein fractions. The amino acid sequence of this protein is believed to be altered in a different way in each of a number of closely linked mutants of the virus. The locus of each of these mutations on a genetic map has been determined by Dr. George Streisinger. Determination of the precise nature of the changes in protein structure resulting from such a series of mapped genetic mutations should ultimately lead to a better understanding of the way in which the "genes" function in the control of protein synthesis.

2. Development of methods for the study of protein structure: In order to make amino acid sequence determinations possible in large protein molecules, such as the bacterial virus serum blocking antigen, a relatively simple method has been developed which permits the separation of up to one hundred peptides obtained from enzymatic digests of proteins. The method, based on two-dimensional electrophoresis and chromatography, seems to be working well and is now being applied to the genetic problem.

3. Isolation of a bacterial virus enzyme and characterization of the enzyme as a lysozyme: Certain bacterial viruses contain an enzyme capable of degrading host cell walls. This enzyme is also found in free form in lysates of cells infected with the virus, but is not present in lysates of uninfected cells. A convenient assay system has been devised for this enzyme and a procedure has been developed which permits isolation of the enzyme in what appears to be nearly pure form. The enzyme was previously considered to be proteolytic since, among other compounds, amino acids are released by its action. However, studies of the purified enzyme indicate that it is a lysozyme rather than a proteolytic enzyme. The following list includes some of the interesting features of this bacterial virus enzyme:

a. Bacterial virus lysozyme appears to be synthesized under the genetic control of the virus. Since the enzyme seems to be relatively low in molecular weight it may ultimately prove to be particularly useful for the study of genetic control of protein synthesis.

b. There are certain similarities between this enzyme and the one believed to be present in a number of mammalian viruses (e.g. influenza). A study of this type of enzyme should shed considerable light on the mechanism of viral infection.

c. A comparison of the structure of this enzyme and lysozyme from other organisms (e.g. other viruses, egg white lysozyme, etc.) should provide information about the species differences of proteins with similar activities.

d. The enzyme lyses E. coli (gram negative) under conditions where egg white lysozyme is ineffectual as a bactericidal agent.

e. It is one of the very few enzymes found in viruses.

Serial No. NHI-117

Significance to Heart Research: This work is part of the continuing effort of the laboratory to gain a greater understanding of the structure, biosynthesis and function of protein molecules, particularly those having enzymatic properties. The work in general is of a highly basic and theoretical sort with obvious bearings on tissue metabolism in general.

Proposed Course of Project: Certain physical, chemical, and biological activities of the proteins which have been isolated should be studied in order to establish purity, and to gain other useful information. Experiments are continuing in an effort to elucidate the effect of genetic mutations on the structure of these proteins in the hope that we will eventually be able to compare fine structure genetic maps with maps of alterations in protein structure.

PART B included: Yes X No

Serial No. NHI-117

PHS--NIH
Individual Project Report
Calendar Year 1958

PART B: Honors, Awards, and Publications

Publications other than abstracts from this project:

G. Koch and W. J. Dreyer, Characterization of an Enzyme of Phage T2 as a Lysozyme, Virology, 6, 291-293 (1958).

Serial No. NHI-118

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS--NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Application of the peptide separation methods to the study of muscle proteins

Principal Investigators: W. Wayne Kielley
William J. Dreyer

Man Years: (calendar year 1958) Patient Days: None
Total 4/15
Professional: 4/15

Project Description:

Objectives: In an effort to test the applicability of the peptide fractionation method to the study of large proteins, to further improve the method, and to attempt to answer some long standing questions concerning muscle proteins, a collaborative project with Dr. W. W. Kielley has been undertaken.

The following list briefly indicates the sort of information we hope to gain:

a. Various proposed relationships between certain of the muscle proteins are found in the literature. The peptide separation method offers the possibility of comparing the chemical structure of such proteins as tropomyosin, actin, myosin A, myosin B, L meromyosin and H meromyosin in order to determine the exact relationship, if any between them.

b. Certain of these proteins may be present in the form of polymers. The peptide separation method should be useful for the determination of the true monomer molecular weight of these proteins.

c. The method may be useful for the study of the "active center(s)" of myosin.

It is assumed that such studies may provide information useful in the eventual elucidation (at the molecular level) of the mechanism of muscular contractions.

Methods:

Preliminary results indicate a monomer molecular weight of 100,000 (plus or minus c.a. 20,000) for myosin. Since the literature value for the molecular weight of myosin as determined by conventional methods is 400,000, this result suggests that myosin as it exists in solution is composed of 3-5 identical subunits of about 100,000 M.W. each. It is hoped that it will be possible to determine the number of subunits with greater precision by developing the method further. A monomer molecular weight of c.a. 80,000 was obtained for actin (literature value = 70 to 80,000). Tropomyosin gave a monomer value close to the reported value (40,000). These preliminary results also indicate that, contrary to certain proposals found in the literature, each of the above proteins is a dintinct entity.

Attempts have been made to study the peptides containing chemically blocked SH groups. However, due to technical difficulties no results are available at present.

Significance to Heart Research: There is no immediate significance to heart research, though more extensive knowledge of the proteins involved in muscular contraction may be of eventual importance.

Proposed Course of Project: The results indicated above are incomplete and should therefore be confirmed and extended by further studies. Several possible means of improving the methods used should be examined. Since the SH groups of the myosin molecule appear to be implicated in its catalytic activity, they should be examined in some detail. Identification of SH containing peptides in proteolytic digests of myosin should be possible.

PART B included: Yes No X

Serial No. NHI-119
1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Investigation of structural and functional relationships in ribonuclease.

Principal Investigator: Frederick H. White, Jr.

Other Investigators: None

Man Years (Calendar year 1958): Patient days: None
Total: 1
Professional: 1
Other: None

Project Description:

Objective: To investigate the relationship between disulfide bridges in ribonuclease and enzyme activity for the purpose of obtaining more information on structural and functional relationships in enzymes.

Methods Employed: Further details of methodology have been elaborated in connection with reduction of disulfide bridges in ribonuclease and reoxidation of reduced ribonuclease. Advances have been made in relating enzymatic activity to specific features of tertiary structure in ribonuclease.

Major Findings. A. A correlation has been made between the concentration of the mixed disulfide, B-carboxy, B-aminoethyl, carboxymethyl disulfide, and the level of reduction of ribonuclease. This mixed disulfide arises as a result of the partial reduction of a disulfide bridge by thioglycolic acid. It has been established as a complicating factor which contributes to the heterogeneity of the reduced protein and is of importance if isolation work is to follow the reduction step.

B. A technique has been developed to test for the homogeneity of partially reduced ribonuclease with respect to the absence of specific disulfide bridges.

C. The disulfide bridge in ribonuclease connecting half-cystine residues 1 and 6 (counting from the N-terminal end of the molecule) has been found not to be associated with the active center of ribonuclease.

D. The reduction methods developed in this work have been successfully applied to lysozyme, an enzyme which resembles ribonuclease insofar as it consists of a single peptide chain of approximately the same length as that of ribonuclease.

E. The reoxidation of reduced ribonuclease has resulted in complete restoration of the enzymatic activity that was lost on reduction. Preliminary physical studies of the reoxidized enzyme have indicated many structural similarities between it and the native enzyme.

F. It has been demonstrated that the thioester derived from thioglycolic acid completely destroys ribonuclease activity and, when present during the reduction of ribonuclease with thioglycolate, contributes to the sulfhydryl content of the reduced protein. Therefore the importance of eliminating the thioester from thioglycolic acid and of inhibiting its formation during reduction of proteins in general has been established.

Significance to Heart Research: This work is part of the continuing effort of the laboratory to gain a greater understanding of the structure, biosynthesis and function of protein molecules, particularly those having enzymatic properties. The work in general is of a highly basic and theoretical sort with obvious bearings on tissue metabolism in general.

Proposed Course of Project: Further comparative studies of native, reduced, and reduced-reoxidized ribonucleases are planned to gain further information on the structural features essential for enzymatic activity of ribonuclease. To facilitate this study, efforts will be made to purify samples of reduced and reduced reoxidized ribonuclease, employing tests homogeneity which have been developed in this laboratory.

Part B included Yes X

Serial No. NHI-119

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

F. H. White, Jr.and C.B. Anfinsen, On the Order of Disulfide Bond Reduction in Ribonuclease, Symposium on Sulfur in Proteins, in press.

M. Sela, F.H. White, Jr., and C. B. Anfinsen, The Reductive Cleavage of Disulfide Bonds and its Application to Problems of Protein Structure, Biochim. et Biophys. Acta, in press.

F. H. White, Jr. and C. B. Anfinsen, Some Relationships of Structure to Function in Ribonuclease, Ann. N.Y. Acad. Sci., in press.

Serial No. NHI 120
1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Studies on the Controlled Degradation of Ribonuclease with Proteolytic Enzymes.

Principal Investigators: C.B. Anfinsen, W. F. Harrington

Other Investigators: Juanita Cooke and Ann Ginsburg, Technical

Man Years (calendar year 1958): Patient Days: None
Total: 1-1/3
Professional: 1/3
Other: 1

Project Description:

Objective: To investigate the action of pepsin and other proteolytic enzymes on bovine ribonuclease under different conditions of pH and temperature with the purpose of preparing and isolating active derivatives of the enzyme which might lack portions of the native structure.

Methods employed: Studies employing the pH stat and chromatographic analysis suggest that pepsin shows a different specificity on ribonuclease as a substrate when digestions are carried out at different pH values. When pH values are in the neighborhood of 1.8, ribonuclease loses a tetrapeptide fragment from the C-terminal end and is inactivated. In the higher pH however, in the neighborhood of pH 2.5, a series of amino acids are split off from the N-terminal end of the molecule and the product appears to be fully active.

Significance to Heart Research: This work is part of the continuing effort of the laboratory to gain a greater understanding of the structure, biosynthesis and function of protein molecules, particularly those having enzymatic properties. The work in general is of a highly basic and theoretical sort with obvious bearings on tissue metabolism in general.

Proposed Course of Project: [illegible] results must be carefully checked by chromatographic analysis of the digestion mixtures at various pHs. The results already suggest, however, that the shape of the molecule is greatly influenced in solution by hydrogen ion concentration and that certain segments of the molecule which are unavailable to attack by pepsin at neutral or slightly acidic pH values may become released from the main structure of the protein at lower pH values and thus be vulnerable to proteolysis.

Part B included Yes No X

Serial No. NH-421
1. Laboratory of Cellular Physiology
2. and Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project title: Proteolytic fragmentation of the myosin molecule

Principal Investigators: Elemer Mihalyi
William F. Harrington

Man Years: (Also given in report of Elemer Mihalyi, Section on Metabolism, Laboratory of Cellular Physiology and Metabolism)

Total: 1.5 Patient Days: None
Professional 1.0
Technical 0.5

Project Description: Myosin is a very large molecule, much too large to be attacked by structural investigations in its intact form. It was thought that the proteolytic fragmentation of myosin, since it yields well defined fragments, may be used advantageously in structural investigations, especially to establish connections between the known functions and certain localized areas of the molecule. It was hoped that the proteolytic process itself might help to clarify the structure of the molecule.

SEE ANNUAL REPORT OF DR. MIHALYI, REFERRED TO ABOVE.

PART B included NO

Serial No. NHI-122
1.Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda,Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Studies on the structure of lysozyme

Principal Investigator: Arnold M. Katz

Other Investigators: None

Man Years (Calendar year 1958) Patient Days: None
Total: 1/2
Professional: 1/2
Other: None

Project Description:

Objective: To isolate;and identify, by two-dimensional paper chromatography and electrophoresis, the peptides from a tryptic digest of reduced, alkylated lysozyme.

Methods Employed: A new technique, for the precise separation of peptides has evolved through work in this department and elsewhere. Its applicability in the determination of the amino acid sequence of a protein enzyme of 15,000 molecular weight is being investigated as a model for future work. Crystalline lysozyme is reduced, alkylated and then digested with trypsin to give a reproducible mixture of peptides. This mixture is resolved into individual peptide spots by chromatography, followed by electrophoresis on large sheets of filter paper. The resulting pattern of peptides is called a "fingerprint" and is quite characteristic of this protein. It has been possible at this time to identify eight of the seventeen peptides expected in this digestion mixture.

Significance to Heart Research: This work is part of the continuing effort of the laboratory to gain a greater understanding of the structure, biosynthesis and function of protein molecules, particularly those having enzymatic properties. The work in general is of a highly basic and theoretical sort with obvious bearings on tissue metabolism in general.

Proposed Course of Project: Further work is directed to the successful identification of the entire complement of peptides from the tryptic digest of lysozyme. This is expected to demonstrate the usefulness of the "fingerprint" method for analytic work and permit its application to the many problems in the study of protein structure and its genetic determination now being studied in this department and elsewhere.

PART B included No

Serial No. NHI-123
1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Studies on lipoproteins.

Principal Investigator: Martin Rodbell

Other Investigators:; None

Man Years (Calendar year 1958) Patient days: None
Total: 1
Professional: 1
Other: None

Project Description:

Objective: To study the structure, function, and metabolism of lipoproteins with particular reference to the role of lipoproteins in fat absorption and transport.

Methods Employed: Lipoproteins, including chylomicrons, are isolated from the plasma and lymph of dogs and humans by centrifugation techniques. After delipidation of the chylomicrons and high-density lipoproteins with chloroform-methanol, the proteins are dissolved in concentrated urea. The urea-soluble proteins are separated by electrophoresis in urea as well as buffer solutions. Incorporation of carbon^{14}-labeled amino acids into the proteins of chylomicrons and high density lipoproteins is followed by conventional techniques for carbon^{14} counting after separating the proteins by paper electrophoresis and extracting the proteins from the paper. The proteins are digested with proteolytic enzymes and the resultant peptides are separated by chromatography and high voltage electrophoresis to form "fingerprint" patterns of peptides. Proteins are analyzed for N-terminal amino acids by a dinitrophenylation technique.

Major Findings.

1. a. N-terminal analyses of dog and human chylomicrons show that at least two proteins are associated with the chylomicrons. One of the proteins from the chylomicrons contains N-terminal aspartic acid (A protein), the same as found for the high-density lipoproteins. The other protein contained N-terminal serine in human chylomicrons and N-terminal glutamic in dog chylomicrons (B protein).

b. The electrophoretic mobility of one of the chylomicron proteins (A protein) is identical to that of the protein isolated from the high-density lipoproteins isolated from both human and dog plasma.

c. The "fingerprint" pattern of the A protein in chylomicrons is identical to that found for the high-density lipoprotein protein. It is

(Major findings - continued)

concluded that the chylomicrons isolated from dogs and humans contain the high-density lipoprotein protein.

d. The solubility properties of the B protein from both dog and human chylomicrons are similar. The "fingerprint" patterns of these proteins are completely different from the high-density lipoprotein protein as well as the plasma low-density (Sf 0-20) protein in humans and dogs. This evidence, coupled with previous findings that human plasma contains A lipoproteins with N-terminal serine (the same as that found for the B protein in human chylomicrons) suggest that this protein (or lipoprotein)is fundamental for fat transport.

2. a. In vivo studies on incorporation of labeled amino atids into the proteins of the chylomicrons isolated from dog lymph during the absorption of fat demonstrate that there is a rapid increase in the specific activities of the two major chylomicron proteins. The specific activity of the A protein in chylomicrons is identical to the specific activity of the high-density lipoprotein in lymph but considerably higher than that found in the plasma high-density lipoprotein fraction. This suggests that the plasma high-density lipoprotein is not in equilibrium with the chylomicron A protein or the lymph high-density lipoprotein.

b. In vitro studies on the incorporation of labeled amino acids in chylomicron proteins by intestinal mucosal cells indicate that the intestinal cells are capable of synthesizing proteins resembling the A and B proteins of chylomicrons. The implications of this study are that the chylomicron protein may be important for the absorption of fat in the intestine and that one of the sources of the high-density lipoproteins in plasma may be the intestine.

c. After the injection of chylomicrons containing labeled A and B proteins into recipient dogs, a rapid disappearance of the chylomicrons and the radioactivity occurs. The A protein activity appears immediately in the high-density lipoprotein fraction indicating equilibration whereas the B protein activity does not appear in the plasma until after the initial rapid disappearance of the chylomicrons. This suggests that the B protein is metabolized along with the chylomicron fat. It is possible that the A protein is also removed with the chylomicrons.

Significance to Heart Research: Abnormal metabolism of lipoproteins is associated with many circulatory diseases. There is a tremendous void in our knowledge of the metabolism of these compounds which must be filled if we are to understand the abnormalities which arise.

Proposed Course of Project: 1. The finding that the proteins of the chylomicrons and the high density lipoproteins can be solubilized in aqueous

(Proposed course of project - continued)

systems after removing the lipids makes it possible to attempt the reconstruction of these lipoproteins in vitro. The interaction of phospholipids with the proteins will be studied with the goal of determining the binding sites on the proteins for lipids and the structure of the resulting "artificial" lipoproteins. These studies may give some knowledge as to the mechanism by which lipoproteins are synthesized in vivo.

2. Further metabolic experiments in the dog will be performed in order to locate the site (or sites) of synthesis of the chylomicron proteins, and the high-density lipoproteins, with emphasis on the role of the intestine in the synthesis of chylomicrons and the function of the lipoprotein in this process. Experiments will be done with isolated tissues on the metabolism of chylomicrons in an effort to determine the fate of chylomicron proteins as well as the associated fat.

Part B included Yes

Ser. No. NHI-123

PHS-NIH
Individual Project Report
Calendar Year 1959

PART B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

M. Rodbell, The Protein and Lipid Composition of Human Chyle and Plasma Lipoproteins, Science, 127, 701 (1958).

M. Rodbell, D.S. Fredrickson, On the Nature of Chylomicron Proteins J. Biol. Chem., in press.

M. Rodbell, D.S. Fredrickson, and K. Ono, The Metabolism of Chylomicron Proteins, J. Biol. Chem., in press.

Serial No. NHI-124

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: The identification of the disulfide bridges in ribonuclease which are essential for activity.

Principal Investigators: C. B. Anfinsen, Edgar Haber.

Other Investigators: None

Man Years (calendar year 1958): Patient days: None
Total: 1
Professional: 1
Other: --

Project Description:

Objective: These studies, which are closely related to those reported by Frederick H. White, Jr., aim to determine the identity of the disulfide bridges which remain uncleaved in solutions of ribonuclease produced by partial reduction of cystine-disulfide bonds. Techniques have been worked out for the fingerprinting of trypsin digests with such partially reduced material and these techniques will be used to study the kinetics of reduction of each bridge. Further details may be found in the report by Dr. Frederick H. White, Jr.

Significance to Heart Research: This work is part of the continuing effort of the laboratory to gain a greater understanding of the structure, biosynthesis and function of protein molecules, particularly those having enzymatic properties. The work in general is of a highly basic and theoretical sort with obvious bearings on tissue metabolism in general.

PART B included Yes No X

Serial No. NHI-125

1. Laboratory of Cellular Physiology and Metabolism
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Studies on the Species Differences and Structures of Ribonuclease.

Principal Investigators: C.B. Anfinsen, Stig E.G. Aqvist, Trygve Tuve

Other Investigators: Juanita Cooke, Technical.

Man Years (Calendar year 1958): Patient days: None
Total: 5/6 year
Professional: 1/3
Other: 1/2

Project Description:

Objective: To isolate and determine the structure of ribonucleases from a variety of biological sources. These studies have been undertaken since it seems a reasonable working hypothesis that the same general structure may occur in the active centers of all of the ribonucleases, but that variations in structural detail may exist in those parts of the structures which are not directly concerned with catalysis.

Methods employed: Sheep pancreas has been examined for its ribonuclease content and four distinct protein components have been isolated from extracts of this tissue by column chromatography. All four seem to be closely related as regards molecular weight and end groups. The structure of the major one of the four components has been compared with the structure of the bovine enzyme and three changes in amino acid sequence have been found. Similar studies are now in progress on ribonuclease from pork pancreas and from spinach leaves.

Significance to Heart Research: This work is part of the continuing effort of the laboratory to gain a greater understanding of the structure, biosynthesis and function of protein molecules, particularly those having enzymatic properties. The work in general is of a highly basic and theoretical sort with obvious bearings on tissue metabolism in general.

Proposed Course of Project: Should the structural studies above indicate the presence of a common constellation of amino acids in the several ribonucleases, the enzyme would probably be isolated from a few other biological sources to extend this "common denominator" approach to the mechanism of catalysis.

Part B included Yes X No

Serial No. NHI-125

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B Honors, Awards, and Publications

Publications other than abstracts from this project:

C. B. Anfinsen, Stig E.G. Aqvist, Juanita P. Cooke and Borje Jonsson, A Comparative Study of the Structures of Bovine and Ovine Pancreatic Ribonucleases, J. Biol. Chem., in press.

Stig E.G. Aqvist and C. B. Anfinsen, The Isolation and Characterization of Some Ribonucleases from Sheep Pancreas, J. Biol. Chem., in press.

Honors and Awards relating to this project: None

Form No. ORP-2
Oct. 1957

PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
Laboratory of Cellular Physiology and Metabolism
Enzymes Section

Estimated Obligations for FY 1959

Total: $261,776

Direct: $173,000

Reimbursements: $ 88,776

Serial No. NHI-126
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: (1) The enzymatic synthesis of "energy-rich compounds and their utilization in biosynthetic reactions.

(2) Heterocyclic Compound Metabolism

Principal Investigator: Roy Vagelos

Other Investigator:

Cooperating Units

Man Years (calendar year 1958):

Total: 2.3
Professional: 1
Other: 1.3

Project Description:

Propionate metabolism: The study of the metabolism of propionic acid in microorganisms has been continued. The fate of lactyl CoA was studied; it was shown that extracts of C. propionicum and C. kluyveri contain a deacylase that hydrolyzes the thiolester. Further metabolism was not investigated.

Methods and Major Findings:

Propionic acid is oxidized by extracts of C. kluyveri by the following sequence of reactions:

Propionyl CoA ⟶ acrylyl CoA ⟶ β-Hydroxypropionyl CoA

⟶ malonyl semialdehyde CoA ⟶ malonyl CoA ⟶ acetyl CoA + CO_2

All the reactions through the formation of malonyl CoA have been demonstrated by partial purification of the enzymes and isolation of the intermediates. The decarboxylation of malonyl CoA has not yet been well demonstrated, though some experiments have suggested that the reaction occurs.

Part A. con't

Heterocyclic Compound Metabolism:

Organisms have been isolated by enrichment culture on numerous pharmacologically active heterocyclic compounds. At present, morphine has been selected for immediate study. The organisms that have been isolated are being tested for their ability to degrade morphine; at the same time, poppy plants have been planted at the agricultural station in Beltsville, Md. Since recent studies have shown that the plant can incorporate α-C^{14}-DL-tyrosine and phenylalanine into the morphine molecule intact while growing on artificial media, it is hoped that cell free extracts can be made of the poppy capsules with which to study the mechanism of biosynthesis at the enzyme level.

Proposed course of Research

All the above work will be continued. It is hoped that the decarboxylation reaction (malonyl CoA to acetyl CoA) will be demonstrated and shown to be reversible. If that is the case, the possibility of studying the role of biotin in carboxylation reactions will be investigated. Also, if good preparations of malonyl CoA can be obtained, the role of malonyl CoA in fatty acid biosynthesis will be investigated (as suggested by Dr. R. Brady).

Morphine degradation by microorganisms and biosynthesis by P. somniverum will be studied in collaboration with Dr. E. Kravitz. Intermediates will be identified and individual reactions will be studied.

Part B Included Yes X No

Serial No. NHI-126

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B.

Publications:

1. Stadtman, E. R., and Vagelos, P. R., Propionic Acid Metabolism, International Symposium on Enzyme Chemistry, Tokyo and Kyoto, (1957), in press.

2. Vagelos, P. R., Earl, J. and Stadtman, E. R., Propionic Acid Metabolism, I. The Purification and Properties of Acrylyl Coenzyme A aminase, J. Biol. Chem., in press.

3. Vagelos, P. R., Earl, J. and Stadtman, E. R. Propionic Acid Metabolism, II. Enzymatic Synthesis of Lactyl Pantetheine, J. Biol. Chem., in press.

Serial No. NHI-127
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH

Individual Project Report
Calendar Year 1958

Part A

Project Title: The Microbiological Degradation of Riboflavin

Principal Investigator: E. R. Stadtman
P. Z. Smyrniotis
T. Miles

Other Investigator

Cooperating Units:

Man years

Patient Days: None

Total:	2.3
Professional:	2.
Other:	0.3

Project Description:

Objectives: The immediate aim of this research is to determine the enzymatic mechanism of riboflavin oxidation to Co_2 and NH_3. It is believed that a detailed knowledge of this biochemical process will reveal basic patterns of metabolism involved in the biochemistry of heterocyclic compounds in general.

PHS - NIH
Individual Project Report
Calendar Year 1958

Methods and Major Findings:

Several intermediates in the breakdown of riboflavin have been isolated in 100mg to 2.0 gram quantities as pure crystalline compounds. The first detectable intermediate has been identified as 1-ribityl-2,3-dioxo-1,2,3,4-tetrahydro-6,7-dimethylquinoxaline (compound I). Its formation from riboflavin results from an oxidative change of the pyrmidine ring with the stoichiometric elimination of one mole of urea and one additional carbon atom (probably Co_2). This conversion presents an interesting problem since the most reasonable intermediates have been synthesized (see report of T. Miles) and were found not to be metabolized by the bacterial system. Compound I has been shown to be converted to a second intermediate (compound II) by a synthetic reaction that involves the addition of two carbon atoms and one nitrogen atoms probably to the ribityl side chain. Compound II is subsequently converted to 3,4 dimethyl-α-pyrone carboxylic acid by a series of unidentified reactions.

The existence of a second pathway of riboflavin catabolism is revealed by the isolation of degradation product that are oxidized to Co_2 and NH_3 without the intermediate formation of the α-pyrone carboxylic acid. The possibility that this second pathway represents a reversal of the biosynthetic pathway is suggested by the observation that one of the compounds isolated will replace riboflavin as a growth factor for a riboflavin requiring strain of Lactobacillus.

Significance:

Proposed course of Research

The isolation and characterization of riboflavin degradation products will be continued. Experiments to determine the detailed enzymatic mechanism of formation of compound I and II will be carried out.

Part B included Yes X No

Serial No. NHI-127

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B:

Publications:

1. Isolation and Structure Proof of 3,4-Dimethyl-6-carboxy-β-pyrone as a Bacterial Degradation Product of Riboflavin. J. Am. Chem. Soc., 80, 2541, (1958).

2. Intermediates in the Decomposition of Riboflavin, Bact. Proc., (1958).

3. The Biosynthesis and Degradation of Riboflavin, Proceedings of the IV The International Congress of Biochemistry, 1958.

4. Bacterial Degradation Products of Riboflavin III. Isolation, Structure Determination, and Biological Transformations of 1-Ribityl-2,3-diketo-1,2,3,4-tetrahydro-6,7-dimethylquinoxaline. J. Am. Chem., Soc. (in press).

Serial No. NHI-128
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: 1. Mechanism of Enzymatic Formation of Threonine from O-phosphohomoserine.

2. Enzymatic formation of cystathionine from homoserine.

Principal Investigator: Martin Flavin

Other Investigator:

Cooperating Units:

Man Years

Total:	2.3
Professional:	1
Other:	1.3

Project Description:

Objectives: Earlier work had shown that the last step in the major pathway of threonine biosynthesis in yeast and E. coli consisted in the isomerization of homoserine to threonine. Although this reaction may have some analogy to the citrate-isocitrate interconversion, Watanabe and Shimura had shown that it also contained a novel feature. They found that, in yeast, the reaction proceeded in at least 2 steps, the first being a phosphorylation at the expense of ATP to yield O-phosphohomoserine, and the second the formation of threonine with the liberation of orghophosphate.

While the seemingly needless expenditure of ATP in the overall reaction sequence might be understandable energetically as a device to shift the equilibrium in favor of the accumulation of a protein building block, the manner in which phosphate might participate in the mechanism of the second reaction raises many questions. The mechanism of conversion of phosphohomoserine to thereonine is of further interest because, although it seems to have no established precedent, it may be analogous to, and serve as a useful model for, an early step in steroid biosynthesis.

We have chosen Neurospora crassa for the investigation of this problem, because of the availability of mutant strains, which may in some cases offer advantages as enzyme sources. Later, they may alos permit more general investigations of the manner of formation and activation of the enzymes being studied.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A con't.

Project Description con't.

The biosynthesis of cystathionine from homoserine and cysteine has never been shown in cell-free preparations, nor have we so far been able to show it in Neurospora. We feel that the mechanism of this reaction may be more complex than a simple elimination of water, and that it can be conveniently studied together with threonine biosynthesis. For example, phosphohomoserine can be tested as a possible intermediate in cystathionine biosynthesis.

Methods and Major Findings

A reliable, accurate assay for threonine has been developed, which requires 5 minutes as compared with 3 or 4 hours for assays previously available.

One gram of pure O-phosphohomoserine has been isolated from an enzymatic incubation of ATP and homoserine. The methods of isolation and of purification of yeast homoserine kinase were modifications of those of Watanabe and Shimura.

Of many unsuccesful attempts to prepare O-phosphohomoserine chemically, the most promising till now has been via N-carbobenzoxyhomoserine —— N-carbobenzoxyhomoserine lactone —— N-carbobenzyoxyhomoserinebenzylamide. Small amounts of phosphohomoserine have been obtained by treatment of the latter with polyphosphoric acid followed by acid hydrolysis. It is planned to try more gentle phosphorylating agents, such as tetra-p-nitrophenylpyrophosphate.

The results of Watanabe concerning the pathway from homoserine to threonine in yeast have been confirmed. The enzyme converting phosphohomoserine to threonine has been identified in Neurospora wild typ 5297, and has been purified 200 fold in a 3-step proceedure. This enzyme activity is undetectable in extracts of mutant 35423, which requires exogenous threonine for growth. Curiously, it has so far been impossible to show the presence of homoserine kinase in Neurospera.

Pyridoxal phosphate has been shown to be a cofactor for the enzyme converting phosphohomoserine to threonine.

The first studies of the mechanism of the latter reaction have been carried out by incubating partially purified enzyme with substrate and cofactor in Tritium-labeled water. If threonine were formed through an olefinic intermediate such as vinylaglycine, it

PHS - NIH
Individual Project Report
Calendar Year 1958

Methods and Major Findings con't

should aquire one atom of hydrogen per mole from the solvent, stably bound to the gamma carbon. It was found, however, that the threonine formed at the end of various periods of incubation had aquired only 0.05 to 0.1 atoms of H per mole from the solvent, barring isotope selection. In addition, threonine itself aquired 0.05 atom of H from the solvent, on incubation with enzyme and cofactor. These results provide fairly strong evidence against an olefinic intermediate. A puzzling finding has been that residual unreacted phosphohomoserine has a 10 x higher tritium concentration than the threonine formed during the incubation.

Proposed Course of Research

We plan to continue the tritium experiments and, at first, to determine the positions into which it is introduced enzymatically in threonine and phosphohomoserine. Later studies will deal with partial reversibilities, testing of possible intermediates, which are available, and search for others with carbon or phosphorus-labeled phosphohomoserine.

From cellulose chromatography it seems likely that a single enzyme catalyzes the complex conversion of phosphohomoserine to threonine. It is fortunate that we have been able to achieve high purification of this enzyme, since this may be essential to a study of its mechanism of action, if intermediates occur only bound to the enzyme surface. Eventually, the enzyme way also be a favorable one with which to study the general manner of binding and mechanism of action of pyridoxal phosphate.

A number of experiments will become possible if phosphohomoserine can be made chemically in large amounts; for example, the investigation of model, non-enzymatic reactions.

As indicated above, we plan to continue work on the enzymatic formation of cystathionine and phosphohomoserine in Neurospora.

Part B Included Yes X No

Serial No. NHI-128

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B

Publications

1. Metabolism of propionic acid in Animal Tissues. I. Enzymatic Conversion of Propionate to Succinate. Martin Flavin and Severo Ochoa, J. Biol. Chem., 229, 965 (1957).

2. Metabolism of Propionic Acid in Animal Tissues. II. Pripionyl Coenzyme A Carboxylation system. Martin Flavin, H. Castro-Mendoza, and Severo Ochoa, J. Biol. Chem., 229, 981 (1957).

3. Metabolism of Propionic Acid in Animal Tissues. III. Formation of Succinate. William, S. Beck, Martin Flavin, and Severo Ochoa, J. Biol. Chem. 229, 997 (1957).

Awards

American Society of Biological Chemists Travel Award for the IV International Congress of Biochemistry, Vienna, 1958.

Serial No. NHI-129
Laboratory of Cellular Physiology
Section of Cellular Physiology
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Metobolism of N-onium Compounds and Methyl Donor Compounds in Anaerobic Microbes.

Principal Investigator: Hugh R. Hayward

Other Investigator

Cooperating Units:

Man Years

Total:	1.3
Professional:	1
Other:	.3

Project Description:

To study the fermentation of choline by extracts of a new species of Clos ridium isolated by the enrichment culture technic with regard to ntermediates and cofactors and to study the mechanism of "high energy phosphate bond" synthesis coupled with this fermentation.

To study the condensation of one carbon fragments to compounds such as acetate by anaerobic microorganisms isolated from soil.

Major Methods and Findings

Experiments with growing cultures of the choline organism in the presence of methyl-or chain-labeled C-14 choline confirmed the overall reaction echeme:

$$(*CH_3)_3\overset{+}{N}CH_2CH_2OH + \tfrac{1}{2}H_2O \longrightarrow (*CH_3)_3N + \tfrac{1}{2}CH_3COOH + \tfrac{1}{2}CH_3CH_2OH + H^+$$

The reaction has been studied in lyophylized cells and cell free sonic extracts and the same overall scheme holds. Attempts to study cofactor requirements (in the crude system) have not as yet indicated any obligate requirements, although appreciable stimulation of choline

PHS - NIH
Individual Project Report
Calendar Year 1958

Major Methods and Findings con't

disappearance results from addition of Fe^{++}, orthophosphate and ADP. ATP is readily hydrolyzed in the crude system, so no phosphate esterification has yet been demonstrated. Of considerable interest is the recent finding that in boiled cell extracts which stimulate the disappearance of choline by sonicates is a compound which spectrally behaves identically to animal cytochrome c. Although a role of this compound in the fermentation of choline has not yet been definitely established there is some evidence to suggest it may function in the reaction. If, as currently seems to be the case, this is a cytochrome pigment, it will be the first one reported from a Clostridium.

Experiments with the betaine-fermenting organism in growing cultures using methyl-labeled C-14 betaine revealed that acetate was synthesized in this organism from the methyl groups of betaine. Further, in experiments with $C^{14}O_2$ and cold betaine there was incorporation of isotope into the acetate, confirming the presence in this organism of an active one-carbon fragment metabolism. However, because of difficulties encountered in growing the organism on a large scale and in preparing active dried cells or extracts, attention was directed instead to the study of the choline organism.

Proposed course of Research

Attempts will be made to fractionate and partially purify the extract from the choline organism so as to be able to study more carefully (1) cofactor requirements, (2) intermediates and reaction mechanism, (3) phosphate esterification. Particular attention will be focused on the nature of the cytochrome if a role for it as a cofactor is confirmed.

(2) Eventually attempts will be made to obtain active preparations from the betaine organism and to study this fermentation in detail.

Part B included Yes X No _____

Serial No. NHI-129

PHS - NIH
Individual Project Report
Calendar Year 1958

Publications

1. The Fermentation of Choline by a New Species of Clostridium Containing A Cytochrome Pigment. (in preparation)

Serial No. NHI-130
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Isoprenoid degradation in aerob microorganism.

Principal Investigator: Werner Seubert

Other Investigator

Cooperating Units:

Man years

Total:	1.3	Patient Days:	None
Professional:	1.		
Other:	.3		

Project Description: Study of the mechanism of isoprenoid degradation.

Methods and Major Findings

Isolation of a microorganism which performs the oxydation of citronellol. Since this compound is an isoprenoid of small molecular weight it represents an ideal model to study the basic mechanism of isoprenoid degradation. Studies with dried cells have shown that in the presence of isovaleric acid and citronellol labeled CO_2 is incorporated into acetate and β-hydroxymethylglutaric acid. In dried cells it also has been shown that labeled acetate is incorporated into amino acids protein and an yellow resin which might be a terpene. Isolation and purification procedures for these products have been worked out.

As a result of studies in enzyme preparations it is proposed that the metabolism of citronellol occurs as shown in the enclosed schemme. These steps shown definitely to occur are indicated by the solid arrows whereas broken arrows indicate steps not yet established.

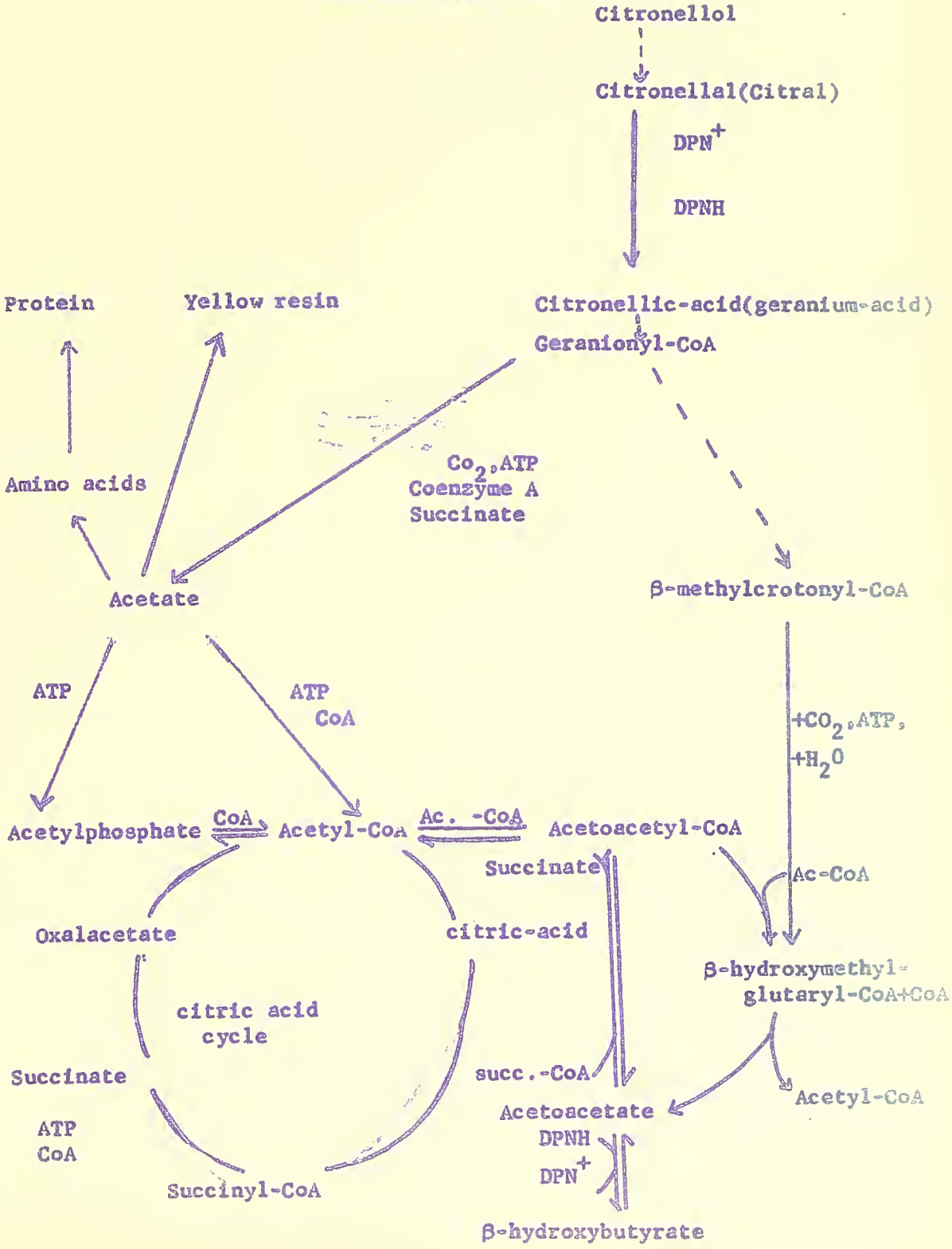
Proposed course of Research

Further studies on degradation of citronellol. Evidences for the conversion of citronellic-acid to β-methylcrotonyl-CoA which is not proved yet. Studies with isoprenoides of higher molecular weight like Ionon and squalene. Chemical characterization of the yellow resin. Study of the synthesis mechanism of this compound.

Part B included Yes No X

Serial No. NHI-130

PHS - NIH
Individual Project Report
Calender Year 1958



Serial No. NHI-131
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

P

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A study of the Metabolism Three-Carbon Compounds in the Anaerobic Microorganism Clostridium propionicum.

Principal Investigator: Howard Goldfine

Other Investigator:

Cooperating Units:

Man Years (calendar year 1958):

Total: 1.3
Professional: 1
Other: .3

Project Description:

To obtain an understanding of the chemical reactions involved in the conversion of alanine to a mixture of acetic and propionic acids. Since these reactions appear to provide the organism with energy for the biosynthetic processes leading to growth, it is hoped that this study will lead to an understanding of the energetics of this organism.

Methods and Major Findings

Strains of this organism, which carry out theaforementioned reactions, were isolated by the process of enrichment culture. A method for obtaining active, cell-free, extracts of these organisms was found and applied. Initial studies revealed that pyruvic acid was not an intermediate in the conversion of alanine to acetic acid in these extracts. However, pyruvic acid is metabolized by these extracts and a major metabolite has been isolated in small quantities. This compound appears to be a dicarboxylic, α-amino acid but is not glutamic or aspartic acid.

Proposed Course of Research

The metabolite of pyruvic acid will be isolated in quantities large enough forpositive identification. The role of this compound in the over-all metabolism will be studied. The conversinn of alanine to acetic and propionic acids will be studied in the light of this new information.

Part B included Yes No X

Serial No NHI-132
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Biochemistry of Differentiation in the Slime Molds.

Principal Investigator: Barbara E. Wright

Other Investigator

Cooperating Units:

Man Years

Total:	2.3
Professional:	1
Other:	1.3

Project Description:

To understand in biochemical terms the morphological changes occuring during differentiation in the slime mold.

Methods and Major Findings.

Acrasin, the chemotactic hormone causing aggregation of the amoebae, may be a steroid-like compound. We have recently investigated two possible modes of action for its biochemical role during differentiation:

(a) An effect on oxygen consumption, since the latter increases following aggregation.

(b) An effect on transhydrogenase, an enzyme mediating electron transfer between TPNH and DPN or DPNH and TPN.

Talalay and Williams-AShman have recently demonstrated such a coenzymatic role for steroid hormones. A shift in the concentration of DPNH might lead to preformed enzymatic changes such as are observed in the slime molds. Negative results were obtained in both these efforts, although a good deal was learned about the metabolism of the slime molds at different stages in their life cycle. It was found for example, that endogenous oxygen consumption could not be enhanced by addition of any substrate tested, which is interesting in view of the requirement of starvation in which is interesting in view of the requirement of starvation in the slime molds as a pre requisite to differentiation.

PHS - NIH
Individual Project Report
Calendar Year 1958

Methods and Major Findings con't

Changes in the activity of various enzymes have been observed, and experiments are in progress to try and understand the mechanism(s) underlying these changes. The behavior of the free amino acid pool, (which is about 10% of the dry weight of the cell), and the breakdown and the synthesis of protein at different stages of development are being investigated. The proteins and amino acids of the amoebae are labeled by growing them on a methionine-requiring mutant of E. coli which had itself been growing on S^{35}-methionine. The distribution of the label is then followed in various chemical fractions of the slime mold at the amoebae, slug and fruit stage. It was found that the specific activity of the methionine in the amino acid pool (based on total ninhydrin) and the proteins (based on biuret) stays constant i.e., methionine is a typical amino acid, forming a constant fraction of the free amino acids and the amino acids in proteins at all stages. It is known that protein is the ultimate source of the carbohydrate (cellulose) formed as an end product of differentiation. Following counts, total ninhydrin, and biuret values it was seen that, as differentiation proceeds, first the amino acid pool falls and remains at 1/3 its initial value, then alcohol-soluble proteins decrease, and finally a fraction of the bulk of the protein (alcohol insoluble, TCA-percipitable) drops between the slug and fruit stages. By following the release of amino acids generally and methionine particularly, it was seen that the greatest proteolytic activity occured after the free amino acid pool had dropped and at the time of the height of various enzymatic activities. In view of the data in the literature concerning relationships of pool levels and the breakdown (and resynthesis) of endogenous proteins, it is conceivable that the decrease in the amino pool of the slime molds brought about by starvation in some way initiates endogenous protein breakdown.

Preliminary experiments show that at the time of maximal proteolysis and enzyme activity there is a maximal uptake of externally supplied S^{35} methionine into protein. It appears, therefore, that "turnover" is enhanced at this period of differentiation.

Serial No. NHI-132

PHS - NIH
Individual Project Report
Calendar Year 1958

Proposed Course of Research

Much more work of the type just discussed is necessary to establish for certain correlations such as proteolytic activity, protein synthesis and maximal enzyme activity. We are then contemplating the fractionation of protein following an exposure to S^{35} methionine at the enzymatically most active (slug) stage of differentiation. A correllation of counts and protein of such an extract will be compared to that of a similar fractionation of methionine-labelled amoebae. It may be possible by such techniques to prove whether actual protein synthesis of specific enzymes at critical times is occuring, or whether some "activiation" mechanism is operating.

Part B Included Yes X No

Serial No. NHI-132

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B

Publications

1. B.E. Wright and M. L. Anderson, Pterine Reductase B.B.A. 28, 370 (1958)

2. B. E. Wright, Folic Acid Coenzyme Forms and Function. Vienna, Vitamin Symposium IV International Congress for Biochemistry (1958).

3. B. E. Wright, Effect of Steroids on Aggregation in the Slime Mold Dictyostelium Discoideum. Bact. Proc. (1958)

4. B. E. Wright and M. L. Anderson, Enzyme patterns during Differentiation in the Slime Mold. McCollum Pratt Symposium in the Chemical Basis of Development, Johns Hopkins 1958. (in press).

Serial No. NHI-133
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH

Individual Project Report
Calendar Year 1958

Part A

Project Title: Synthesis, Isolation and Characterization of Compounds of Biological Interest.

Principal Investigator: H. Todd Miles

Other Investigator

Cooperating Units:

Man Years

Total:	1.3
Professional:	1
Other:	.3

Project Description:

The purpose of the research is to synthesize compounds for use as substrates in enzymatic studies and to attempt the isolation and characterization of biological intermediates.

Methods and Major Findings

The structure of a new bacterial degradation product, isolated by P. Z. Smyrniotis and E. R. Stadtman, was shown by degradative reactions to be 1-ribityl-6,7-dimethyl-1,2,3,4-tetrahydro-2,4-diketoquinoxaline. The new compound has been synthesized chemically by two methods. A number of possible metabolic intermediates in this degradative pathway have also been synthesized.

Preliminary experiments on the structures of three other bacterial degradation products of riboflavin have been carried out, but it is too early to draw definite structural conclusions. One of the compounds appears also to possess the diketoquinoxaline chromophore and to have undergone extensive modification of the ribityl side chain.

The infrared spectra of D_2O solutions of the nucleosides and nucleotides studied earlier have been put on a quantitative basis. The integrated intensities of the peaks in the carbonyl region have been determined by Ramsay's method. The numerical values are independent of concentration.

Serial No. NHI-133
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH

Individual Project Report
Calendar Year 1958

Part A

Project Title: Synthesis, Isolation and Characterization of Compounds of Biological Interest.

Principal Investigator: H. Todd Miles

Other Investigator

Cooperating Units:

Man Years

Total:	1.3
Professional:	1
Other:	.3

Project Description:

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Methods and Major Findings

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The infrared spectra of D_2O solutions of the nucleosides and nucleotides studied earlier have been put on a quantitative basis. The integrated intensities of the peaks in the carbonyl region have been determined by Ramsay's method. The numerical values are independent of concentration.

Part A cont'd

The same method has been applied to solutions of polyadenylic and polyuridylic acids (prepared with the polynucleotide phosphorylase discovered by Grunberg-Manago and Ochoa). It has been found that infrared spectra give a new measure of polynucleotide interaction and that the tautomeric form of the uracil units in the two-stranded helix can be definitely determined and that of the adenine units probably determined.

Proposed Course of Research

Present studies will be continued.

Part B Included Yes X No

Serial No. NHI-139

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B:

Publications:

1. Infrared Spectra and Tautomeric Structure of Nucleosides and Nucleotides in D_2O Solution. II Biochim. Biophys. Acta 27, 76 (1958)

2. Infrared Spectra and Tautomeric Structure in D_2O Solution of Polyadenylic and Polyuridylic Acids. Chem. and Ind. 591 (1958).

3. Quantitative Infrared Spectra in D_2O of Some Nucleosides, Nucleotides, and Polynucleotides: A New Measure of Polynucleotide Interaction. Biochim. Biophys. Acta, in press.

4. Isolation and Structure Proof of 3,4-Dimethyl-6-carboxy-α-pyrone as a Bacterial Degradation Product of Riboflavin. J. Am. Chem. Soc., 80, 2541 (1958). With P. Z. Smyrniotis and E. R. Stadtman.

5. Bacterial Degradation Products of Riboflavin III. Isolation, Structure Determination, and Biological Transformations of 1-Ribityl-2,3-diketo-1,2,3,4-tetrahydro-6,7-dimethyl-quinoxaline. J. Am. Chem. Soc., in press. with P.Z. Smyrniotis and E. R. Stadtman.

Serial No. NHI-134
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Metabolism of Hydrogen in Anaerobic Bacteria (with particular reference to Clostridium kluyveri)

Principal Investigator: Stephen C. Kinsky, Ph. D.

Other Investigator:

Cooperating Units:

Man Years (calendar Year 1958):

Total:	1.3
Professional:	1
Other:	.3

Project Description:

1. Hydrogen gas is consumed or evolved by numerous bacterial species. The oxidation of hydrogen has been studied in cell-free systems by several investigators but these experiments have been conducted mainly with unnatural electron acceptors, e.g. oxidation-reduction dyes. The purpose of the present research is to isolate and identify the naturally occuring cofactors and electron acceptors involved in hydrogen oxidation. The organism chosen for study is the anaerobic bacterium, C. kluyveri.

Methods and Major Findings:

(1) The presence of boiled cell extract is required for pyridine nucleotide reduction with hydrogen.

(2) Boiled cell extract is not required for flavin or dye reduction with hydrogen.

(3) At least two enzymes are involved in pyridine nucleotide reduction.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A. con't

Methods and Major Findings con't.

(4) An enzyme not involved in flavin or dye reduction is required for pyridine nucleotide reduction.

(5) The activity of the boiled cell extract is due to 2 components. One of these has been tentatively identified as FAD (flavin-adenine-dinucleotide). FAD alone is inactive and requires the presence of an additional factor.

Proposed Cource of Research

Present studies are directed towards isolating and determining the structure of the second component. The applicability of paper chromatography, electrophoresis and anion chromatography is in progress.

Part B. included Yes X No

Serial No NHI-134

PHS - NIH
Individual Project Report
Calendar Year 1958

Publications

1. Kinsky, S. "Hydrogen Oxidation in C. kluyveri" Federation Proceedings, 117, 254 (1958); J. Biol. Chem. (in press).

Serial No. NHI-135
Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: A: Intermediary metabolism of amino acids w. particular emphasis on those reactions involving overall reductive deaminations.

B: Anaerobic metabolism of methyl-group donor compounds and N-onium compounds. (see report from Dr. Hugh Hayward)

Principal Investigator: Thressa C. Stadtman

Other Investigator: John Hardman

Cooperating Units:

Man years

Total:	2.3
Professional:	1
Other:	1.3

Project Description:

Objectives: To obtain information concerning the nature of the energy-rich intermediates formed when various amino acids undergo reductive deamination to form the corresponding fatty acids and ammonia. Also to study the electron transfer reactions (anaerobic oxido-reduction reactions) involved in these processes.

Methods and Major Findings

A menadione-dependent phosphatase that was discovered in partially purified glycine reductase fractions prepared from Clostridium sticklandii wa studied in some detail and partially purified. This enzyme proved to be a unique kind of phosphatase and was postulated to be, instead, a transferase which, in the presence of this unnatural quinone, behaved only as a hydrolase. On the basis of this assumption, studies were made on the glycine reductase system regarding the possible participation of a quinone. This is of particular interest for the oxidation-reduction reaction in question leads to the formation of ATP. As judged by a number of criteria such as marked sensitivity to antimycin A, inactivation

Methods and Major Findings cont'd

by irradiation or solvent extraction procedures, inactivation by menadione and related simple naphthoquinones and activation of aged or solvent-treated extracts by vitamin E, a quinone is indded involved in the overall process. This poses the interesting problem as to whether the phosphorylation reaction in the glycine reduction process is coupled to an oxido-reduction reaction at the quinone level. If so, this soluble system may offer possibilities for a study of mechanism ofoxidative phosphorylation at this level.

The fermentation of γ-amino butyrate by a new Clostridium has been pursued by Mr. Hardman and he has succeeded in isolating an active soluble system that catalyzes the overall reaction giving rise to butyrate, acetate and ammonia. He has already outlined the general pathway involved in the metabolism of this compound and has some of the cofactor requirements worked out. He appears to have solublized the energy-yielding system also and has carried out some preliminary purification steps on the enzyme system. Currently he is studying a $DPNH^+$-linked succinic semialdehyde reduction to γ-hydroxy butyrate, one of the steps in the process. In the microorganism, glutamate is the normal electron donor and we wish to examine the possibility that the coupled reaction may provide phosphate-bond energy for the organism.

Significance:

The observation that glycine reduction to acetate by soluble enzyme preparations of clostridium sticklandii is markedly sensitive to antimycin A is of some interest because bacteria in general are resistant to this antibiotic. This appears to be the first example of a bacterial system that is inhibited by antimycin A.

Proposed Course of Research:

Further purification of the glycine reductase system and attempts to identify the natural quinone involved in the overall reaction and its exact role. Is it really oxidative phosphorylation at the quinone level?

In the γ-amino butyrate system the oxido-reduction reactions

(1) Succinic semialdehyde —— γ -oH butyrate coupled with glutamate oxidation
and
(2) oxido-reduction of vinylacetate at the flavin level will be critically examined withrespect to possible phosphorylation mechanisms

Part B Included Yes X No

Publications:

1. The Microbial Metabolism of Steroids. Chapter in "Cholesterol" Ed. by Robert P. Cook Academic Press, Inc. 1958. p. 457.

2. A menadione-dependent enzymic hydrolysis of p-nitrophenyl phosphate. J. Biol. Chem. (in press)

3. The participation of a quinone in the enzymic reduction of glycine by Clostridium sticklandii, Biochemische Zeitschriff. (in press).

4. Ferrous iron dependent alkaline phosphatase of yeast. B.B.Acta (in press).

5. A menadione dependent enzymic hydrolysis of p-nitrophenyl phosphate. Int. Congress fur Biochemie, Wien, 1958.

6. In preparation with J. Hardman
   (1) The fermentation of -amino valerate by Clostridium aminovalericum (N_ov. sp.)

   (2) The fermentation of γ-amino butyrate by Clostridium aminobutyricum (NOv. sp.).

Serial No. NHI-136

Laboratory of Cellular Physiology
Section on Enzymes
Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: The Enzymatic Mechanism of Crotonyl CoA reduction by Diphosphopyridine Nucleotide.

Principal Investigator: E. B. Brown, Jr.

Other Investigator

Cooperating Units:

Man Years

Total:
Professional:
Other:

Project Description:

To study the process of phosphorylation coupled to electron transfer in a non-particulate, cell-free system.

Methods and Major Findings:

Since beginning the research problem in November, 1957 various systems have been studied in which cysteamine and pantetheine analogues of crotonyl-CoA are reduced to the corresponding butyryl compounds catalyzed by enzymes from C. kluyveri. Others have reported that this reduction, which proceeds with a standard free energy change of -14,000 calories, involves phosphorylation coupled to electron transfer. Our studies to date confirm the presence of phosphorylation but cast doubt on the source of the ATP produced. Accumulated evidence favors the occurrence of a dismutation of crotonyl-CoA analogues to acetyl-CoA and the formation of ATP via acetyl phosphate.

Proposed course of Research

An alternate system which avoids the possibility of phosphorylation via acetyl phosphate is under investigation in a continuing effort to study phosphorylation coupled to electron transport.

Part B included Yes_____ NO X

Form No. CRP-2
Oct. 1957

PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
Laboratory of Cellular Physiology and Metabolism
Metabolism Section

Estimated Obligations for FY 1959

Total:	$612,832
Direct:	$281,000
Reimbursements:	$331,832

Serial No. NHI-137
1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Antibodies to Tissue Antigens in Clinical and Experimentally Produced Diseases.
(Project started 1956, not completed)

Principal Investigator: Howard Goodman, M.D.

Other Investigators: James Baxter, M.D., James Allen, M.D.
Robert Bowser, technician

Cooperating Units: John Fahey, M.D., NCI., and J.E. Rall, M.D., NIAMD.

Man Years (calendar year 1958)
Total: 1.5
Professional: 0.75
Other: 0.75

Patient Days (calendar year 1958)
200

Project Description:

Objective: To determine the role of antibodies to tissue antigens in disease states by searching for evidence of such antibodies in patients and by attempting to produce auto-antibodies to tissue antigens in animals.

Methods Employed: Boyden's Tannic Acid Hemagglutination test has been found to be a most sensitive serological test for detecting weak antibody titers to tissue antigens. Complement-fixation and precipitin tests are also employed. Tissue extracts have been saline extracts, or distilled water extracts of nuclei to produce nucleoprotein extracts. Rabbits and rats are immunized by injections of tissue extracts or suspensions incorporated in Freund's adjuvant.

Patient Material and Major Findings:

A. Studies on patient sera: 13 sera from patients with chronic thyroiditis have been found to have antibody titers to human thyroglobulin (prepared by saline extraction of colloid goiter). 19 of 22 sera from patients with lupus erythmatosus have been found to have antibodies to nucleoprotein extracts of calf thymus. No antibodies to saline extracts of human kidney have been found in patients with renal disease. In the sera of patients with thyroiditis and lupus erythmatosus, the antibodies have been found in the gamma globulin fraction in experiments

done in conjunction with Dr. John Fahey of the Cancer Institute. He also has characterized the gamma globulins by di-ethyl-amino cellulose column chromatography, and our tests of the eluted fractions show that the thyroiditis sera have "normal" gamma globulins, while the antibodies against nucleoproteins in lupus sera are in the elution fractions which usually contain macro-globulins.

B. Animal immunization studies: Witebsky and Rose succeeded in producing autoantibodies to thyroglobulin extracts and corresponding thyroid lesions. We have failed to produce detectable antibodies to rabbit nucleoprotein extracts when rabbits were immunized with rabbit nucleoprotein extracts. But when human nucleoprotein extracts were used to immunize rabbits, antibodies were produced not only to human nucleoprotein extracts but also to rabbit nucleoprotein extracts. These rabbits now have circulating gamma globulins which react with their own nucleoproteins - i.e. an experimental analogue of the L. E. factor. So far no evidence of tissue damage has been found.

Significance to Heart Research: These experiments indicate that production in animals of antibodies to foreign nucleoproteins (e.g. bacterial) can produce antibodies which also react with native nucleoproteins, i.e. the experimental analogue of the L. E. factor. If confirmed, this data will provide an experimental basis for the hypothesis that lupus erythematosus is a disease of auto-antibodies, and provide an explanation for the production of such auto-antibodies. Although these experiments were intended originally to test the hypothesis that auto-antibodies are responsible for human glomerulonephritis, so far no evidence of such antibodies has been found, although the search continues.

Proposed Course of the Project: In conjunction with Dr. Fahey (NCI) further studies of the characteristics of the gamma globulins which react with thyroglobulin and nucleoproteins will be carried out. Immunization with nucleoproteins will be continued to see if antibodies to nucleoprotein will produce tissue damage in experimental animals similar to that found in clinical lupus erythematosus. Further attempts will be made to produce auto-antibodies to rat kidney in light of the preliminary successful reports from Dr. Heymann at Western Reserve University.

Part B included - Yes.

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Proposed Course of the Project: In conjunction with Dr. Fahey (NCI) further studies of the characteristics of the gamma globulins which react with thyroglobulin and nucleoproteins will be carried out. Immunization with nucleoproteins will be continued to see if antibodies to nucleoprotein will produce tissue damage in experimental animals similar to that found in clinical lupus erythematosus. Further attempts will be made to produce auto-antibodies to rat kidney in light of the preliminary successful reports from Dr. Heymann at Western Reserve University.

Part B included - Yes.

Serial No. NHI-137

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications:

Publications other than abstracts from this project:

Goodman, H.C. and Baxter, J. H. The Search for Anti-Kidney Antibodies in Patients. Proceedings of the Ninth Annual Conference on the Nephrotic Syndrome, p. 61, 1958.

Honors and Awards relating to this project:

None.

Serial No. NHI-138

1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Clinical Studies on Plasma Protein Metabolism (Started 1956 - not yet completed)

Principal Investigator: Robert S. Gordon, Jr.

Other Investigators: None

Cooperating Units: Dr. Thomas Waldmann, Metabolism Section, NCI

Man Years (Calendar year 1958)		Patient Days (Calendar year 1958)
Total:	.75	250
Professional	.75	
Other:	0	

Project Description:

Objectives, Methods Employed, Patient Material, Major Findings:

Studies with I^{131} labeled albumin and polyvinylpyrrolidone (PVP) have indicated that there exists an abnormal permeability of the gastrointestinal tract in patients suffering from hypercatabolic hypoproteinemia. Such abnormal physiology has been demonstrated in a number of patients with manifest disease of the GI tract regional enteritis, ulcerative colitis, etc.) and also in patients in whom the hypoproteinemia is the only evident disease.

Proposed Course of Project: These studies are being continued and as many patients as possible are being added to the series. In addition, labeled polyvinylpyrrolidone is being made available to qualified investigators throughout the world. It is hoped that the defect responsible for loss of protein into the GI tract in patients who have no recognizable gastrointestinal disease will be discovered.

Significance to Heart Research: Investigation of hypoalbuminemia is of indirect importance to heart research, inasmuch as it will increase understanding of mechanisms of edema formation. In addition a minor degree of loss of plasma protein into the GI tract has been demonstrated in one patient with manifest heart disease and a marked degree of loss in another. It is possible that this process is of significance in the hypoproteinemia associated with certain cardiac conditions (especially constrictive pericarditis).

Part B. included - Yes

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Gordon, R. S., Jr. The Preparation of Radioactive Polyvinylpyrrolidone for medical use. J. Polymer Sci. 31, (No. 122) 191-92, 1958.

Honors and Awards: None

Serial No. NHI-139
1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A.

Project Title: Studies of Inhibitors of Cholesterol Biosynthesis. (Started 1955 - not completed)
Principal Investigator: Daniel Steinberg

Other Investigators: D. S. Fredrickson, J. Avigan, E. B. Feigelson. Hugh Vroman, Technician

Cooperating Units: None

Man Years (calendar year 1958):
Total: .95
Professional: .70
Other: .25

Patient Days (calendar year 1958):
70 days

Project Description:

Objectives: To study the efficacy of various compounds as agents for lowering serum cholesterol level by way of inhibition of cholesterol biosynthesis.

Methods Employed: Rats are fed synthetic diets containing the experimental compound and sacrificed at various time intervals. Serum and tissue cholesterol concentrations are measured. As a measure of the rate of cholesterol synthesis the uptake of C^{14}-labeled acetate or mevalonate is determined just prior to sacrifice. Effects on adrenal steroid production are measured by assay of adrenal vein blood obtained by direct cannulation.

Clinical studies entail measurement of serum lipid concentrations and also periodic evaluation of liver function and kidney function as well as hematologic findings.

Patient Material, Major Findings: (1) The studies on the mechanism of action of α-phenylbutyrate have been completed. They indicate an action on acetate activation and are compatible with the negative clinical results with this compound and with β-phenylvalerate.

(2) The previously reported effects of Δ^4-cholestenone on adrenal function have been confirmed and extended. Simultaneous administration of adrenal steroids prevents the adrenal hypertrophy,

suggesting that overproduction of ACTH by the pituitary is essential for this hypertrophy. Of interest was the finding that there is involution of the prostate and seminal vesicles in male rats receiving high doses of Δ^4-cholestenone but no analogous influence on ovaries and uterus of female rats receiving similar doses. This suggests that cholesterol may be intimately related to androgen synthesis but that estrogen synthesis occurs by way of an independent pathway. Lower doses (0.3% in the diet) have been shown to cause adrenal hypertrophy without impairing growth rate.

(3) Benzmalacene, synthesized by Merck, Sharp & Dohme, and reported by them to be effective in lowering serum cholesterol levels, has been studied in dogs and also at the clinical level. Highly significant depressions of cholesterol levels were obtained in dogs without obvious toxic manifestations. Three patients have received the compound and significant lowering of cholesterol level observed in two. Two patients have had moderately severe gastric distress and all of the patients have shown an elevation of BSP retention which returns quickly to normal on withdrawal of the drug. The mechanism of action is under investigation in rats.

(4) MER-29, synthesized by the William S. Merrell Company, has been studied in rats. A profound lowering of serum cholesterol level has been obtained (from control values of 62 mg.% down to an average of 24 mg.% in 26 days). The liver cholesterol level also falls. No important inhibition of incorporation of C^{14}-acetate or C^{14}-mevalonate could be demonstrated. Attempts to demonstrate an inhibition of cholesterol absorption or cholic acid absorption were negative. The compound has profound toxic effects on the animals, as yet unexplained, and the mechanism of action is under further study. No clinical trials are planned.

(5) Several compounds related to Δ^4-cholestenone have been found to be ineffective as inhibitors: 2-alpha-methyl-cholestenone, 6-alpha-methyl-cholestenone, sitostenone and 3-methoxy-cholesterol.

Significance to Heart Research: In addition to the possibility of uncovering a clinically useful drug for the treatment of hypercholesterolemia, the studies described here may uncover further useful information about the physiologic role of cholesterol.

Proposed Course of Project: Compounds effective as inhibitors of cholesterol synthesis will be studied for possible effects on adrenal function. The mechanism of action of Benzmalacene and MER-29, both very effective in lowering serum cholesterol levels, deserves further study. Those compounds found to be non-toxic in animal experiments will be tested at the clinical level.

Part B included - Yes.

Serial No. NHI-139

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Fredrickson, D.S., Peterson, R.E., and Steinberg, D. Inhibition of adrenocortical steroid secretion by Δ^4-cholestenone. Science 127: 704-705, 1958.

Masters, R. and Steinberg, D. Studies on the mechanism of action of alpha-phenylbutyrate. Biochim. et Biophys. Acta 27: 592-597, 1958.

Steinberg, D., Fredrickson, D.S. and Avigan, J. Effects of Δ^4-cholestenone in animals and in man. Proc. Soc. Expt. Biol. & Med. 97: 784-790, 1958.

Honors and Awards relating to this project:

None.

Serial No. NHI-140
1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies of the mechanisms of fat transport and metabolism. (Project started 1956 - not completed).

Principal Investigator: Donald S. Fredrickson

Other Investigators: Dr. Duncan McCollester (until July, 1958)
Collaborating: Dr. Robert Gordon, Dr. Martin Rodbell, Dr. John Stephenson (LTD-NHI)
Technician: Mr. Katsuto Ono

Cooperating Units: None

Man Years (calendar year 1958)
Total 2.6
Professional: 1.7
Other: 0.9

Patient Days (calendar year 1958)
50

Project Description:

Objectives: The specific problems studied within this project included:

1) Kinetic aspects of transport and metabolism of labeled unesterified fatty acids (UFA) in the plasma.
2) Interrelationships of plasma lipoproteins, with special attention to the protein composition of the chylomicrons.
3) Metabolism of labeled chylomicrons triglyceride fatty acids (TGFA) and the role of plasma UFA transport in this process.

Methods Employed: 1) Unesterified fatty acids tagged with C^{14} in various positions are administered to humans, dogs, or other animals. The changes in concentration of radioactivity in plasma UFA and expired air as $C^{14}O_2$ are measured, and neutral lipids in plasma and tissues are examined for incorporation of radioactivity. Such measurements are being made over a wide range of differences in nutritional and metabolic status, including certain diseases. (Principal collaborator: Dr. Gordon).

2) The chemical composition of the protein moieties of lipoproteins isolated from plasma and lymph from humans and dogs has been studied by electrophoresis, terminal amino acid analyses, and fingerprinting techniques (principally by Dr. Rodbell). Chylomicrons

labeled in the peptide moieties have been obtained from donor animals fed labeled precursors or by in vitro incubation of labeled amino acids with intestinal mucosal cells. The metabolism of these labeled proteins have been observed in recipients.

3) The role of UFA transport in chylomicron TGFA metabolism has been further studied by injection of labeled substrates, prepared from donor animals, into recipients. The fluxes of C^{14} in expired air and in the plasma UFA fraction have been compared with that in dogs given labeled UFA only.

Patient Material, Major Findings: 1) The disappearance rate of UFA from plasma has been found similar for palmitic, oleic and linoleic acids. Based on the earliest phase of disappearance, and with the assumption that all plasma UFA behaves the same, the fractional turnover rate has been found to be dependent on plasma UFA concentration and to vary from 0.1-1.5 mEq/min. The plasma disappearance and rate of oxidation of labeled palmitate carbons-6 and -11 have been compared in two humans and found reasonably similar to that of labeled carbon-1. Labeled $C^{14}O_2$ (bicarbonate) has been found to not appear in plasma UFA, indicating the irreversibility of the oxidative step as far as the tracer kinetic studies are concerned. The disappearance curves of UFA from plasma and the rates of expiration of $C^{14}O_2$ have been adapted mathematically to a theory of kinetic analysis developed by Dr. Stephenson. On this basis a tentative scheme for plasma UFA metabolism was developed, including the interpretation of the late slow decline of radioactivity in blood as representative of "recycling" of UFA into the plasma. Such "recycling" limited the fraction of plasma turnover being directly oxidized. The value of this theoretical approach has been recently demonstrated by the results of an experiment designed to test its underlying assumptions. It was found that increasing the rate of expiration of UFA-C^{14} by 6 to 10 fold in an exercised human did not affect the later recycling phase of the blood disappearance curve in the predicted manner. Hence it has become necessary to further investigate the nature of the labeled UFA in the later portions of these disappearance curves. By cross-transfusion experiments, it has been established that this labeled "UFA" is actually metabolized much more slowly. After chemical extraction of this acidic radioactivity and recombination with albumin it still disappears from blood at a very slow rate. Hence this radioactivity is due either to a conversion of the injected UFA to acidic material quite different in its metabolism, or to a contaminant present in all labeled UFA preparations used and especially prevalent in linoleic acid preparations. Further attempts to identify this acidic material is in progress. By eliminating the radioactivity found in plasma after about 60 minutes, the interpretation of UFA turnover will be simplified, andthe caloric contribution of UFA transported via plasma brought more into agreement with results suggested by other experimental methods.

2) It has been established that the protein moiety of chylomicrons is specific, complex, and related to that in other plasma lipoproteins. In both dogs and humans, one of the polypeptides present is identical to that in the major High Density lipoproteins. Some of the protein in chylomicrons may be synthesized in the gut, since radioactive amino acids are incorporated into these proteins by intestinal mucosal cells in vitro. Two of the labeled chylomicron proteins remain, or reappear in the plasma in other lipoproteins during removal of chylomicrons. These experiments establish for the first time a major relationship between chylomicrons and other plasma lipoproteins and suggest the great importance of certain specific proteins in transport of both exogenous and endogenous fatty acids.

3) We have interpreted experiments with chylomicron TGFA and UFA as supporting the hypothesis that plasma UFA transport is not obligatory in metabolism of the triglyceride fatty acids during chylomicron removal. We believe this still to be true on the basis of considerable evidence, but the kinetic interpretations used as one argument must now be revised in consideration of the recent findings in the tracer UFA experiments mentioned above. These experiments will be reinterpreted when this aspect of normal UFA metabolism has been clarified.

Significance to Heart Research: The metabolism of plasma UFA and of chylomicrons represent two of the quantitatively most important aspects of fatty acid transport, and hence of lipid metabolism in general. Investigations in the latter area remain appropriate to the study of atherosclerosis and cardiac metabolism.

Proposed Course of Project: Work will continue along the above lines with major emphasis on UFA metabolism. The possibility of radically different plasma turnover rates for a small fraction of plasma UFA (and possible conversion of some acids to these more slowly metabolized acids) suggested by recent experiments is of considerable importance to the problem of UFA metabolism in general. When clarified, it may offer an explanation for several heretofore incompatible aspects of our own work and similar tracer studies from other laboratories. With appropriate reservations for the hazards involved in interpretation of such complex data, we hope to eventually program some of these corrected data for a computer to aid calculations in individual studies, especially in humans. Much more effort will be directed to isolation of neutral lipids in plasma and tissues which appear to be involved in the intermediate steps of UFA metabolism. In this regard, we will collaborate with Dr. Avigam in pursuing studies previously begun of metabolism of plasma triglycerides in lipoproteins other than chylomicrons.

Part B included - Yes.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Fredrickson, D.S. and Ono, K., An improved technique for the measurement of Carbon^{14} dioxide in expired air, using the liquid scintillation counter. J. Lab. Clin. Med. 51: 147, 1958.

Fredrickson, D.S., McCollester, D.L., Havel, R.J., and Ono, K. The early steps in transport and metabolism of exogenous triglyceride and cholesterol. Chemistry of Lipids as Related to Atherosclerosis, I. H. Page, Editor, Springfield, Ill., C.C.Thomas, 1958, p. 205.

Fredrickson, D.S., McCollester, D.L., and Ono, K., The Role of Unesterified Fatty Acid Transport in Chylomicron Metabolism, J. Clin. Invest. 37: 1333, 1958.

Fredrickson, D.S. and Gordon, R.S., Jr. The Metabolism of Albumin-Bound C^{14}-Labeled Unesterified Fatty Acids in Normal Human Subjects. J. Clin. Invest. 37: 1504, 1958.

Fredrickson, D.S. and Gordon, R.S., Jr., Transport of Fatty Acids, Physiol. Rev. 38:585-630, 1958.

Honors and Awards:

None

Serial No. NHI-141
1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: 1. Epinephrine Induced Hyperlipidemia .
(Project started Jan. 1, 1958 - not completed)
2. Binding of Unesterified Fatty Acids (UFA) in Plasma.
Principal Investigator: Eleazar Shafrir, Ph.D.

Other Investigators: Daniel Steinberg, Karl E. Sussman and DeWitt S. Goodman.

Cooperating Units: None

Man Years (calendar year 1958)
Total: 2.05
Professional: 1.9
Others: .15

Patient Days (calendar year 1958)
None

Project Description:

Objectives, Methods Employed, Major Findings:

(1) Administration of long-acting epinephrine in dogs was shown to produce marked but transient elevation of plasma UFA and lasting elevation of plasma cholesterol and phospholipids, occurring 24 hrs. after the injection. The elevated lipids appear in major part as 1.019-1.063 lipoproteins. Low density lipoproteins and serum triglyceride levels are not appreciably affected by epinephrine. Relationship between UFA elevation and subsequent lipoproteinemia was studied.

(2) Titration of ultracentrifugally isolated lipoproteins show that the lipoproteins compete with albumin in binding of plasma UFA. The amount bound by the lipoproteins is small in normal human plasma but increases considerably in pathologic conditions in which there is an increase in the concentration ratio of lipoproteins to albumin, particularly in nephrotic syndrome, hyperlipemia and hypoalbuminemia. Measurements of competitive binding were performed in an isolated system composed of low density β-lipoproteins, C^{14}-UFA and albumin. Association constants for low density lipoproteins and several unesterified fatty acids were derived and numbers of classified UFA-binding sites calculated.

Significance to Heart Research: A number of pieces of circumstantial evidence suggest a relationship between unesterified fatty acid metabolism and serum protein levels. Since through such relationships UFA would play a part in determining serum cholesterol levels it is important to explore this matter.

Proposed Course of Project: (1) The mechanism of epinephrine-induced lipidemia is investigated. Effects of adrenalectomy, hypophysectomy as well as in vitro effects of epinephrine on adipose tissue are tested. Relation of lipidemia to the calorigenic effect of epinephrine is being evaluated.

(2) Binding studies in isolated system are continued with high density (α) lipoproteins which appear to bind UFA more tightly than the low density lipoproteins. As the affinity of UFA to lipoproteins seems to increase with the chain length, the distribution of C_{20} and C_{22} UFA between albumin and lipoproteins is being assayed.

Part B included - Yes.

Serial No. NHI-141

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Shafrir, E. Partition of unesterified fatty acids in normal and nephrotic syndrome serum and its effect on serum electrophoretic pattern. J. Clin. Invest. 1958, in press.

Goodman, D.S. and Shafrir, E. The interaction of human low density lipoproteins with long-chain fatty acid anions. J. Amer. Chem. Soc. 1958, in press.

Honors and Awards relating to this project:

None.

Serial No. NHI-142
1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Cross-linking of Proteins with Formaldehyde.
(Project started September, 1957 - completed June 1, 1958)

Principal Investigator: Elemer Mihalyi

Other Investigators: M. I. Knoller

Cooperating Units: None

Man Years (calendar year 1958):
Total: 1.0
Professional: 0.5
Other: 0.5

Patient Days (calendar year 1958)
None

Project Description:

Objectives: To study the mechanism of reaction of formaldehyde with proteins, with special reference to gelation phenomena.

Methods Employed: Kinetic investigation of viscosity and opacity changes, chemical analysis of the formaldehyde treated materials.

Major Findings: Under suitable conditions any protein solution will form a gel on addition of formaldehyde. However, the necessary conditions are widely different with various proteins. Although the number of proteins studied so far is small, two clear-cut classes can be distinguished: (1) fibrous proteins (fibrinogen and myosin were studied), which form a gel at relatively low protein (1.0%) and formaldehyde concentration (1%) with a pH optimum on the alkaline side of the isoelectric point (7.5); (2) globular proteins (bovine serum albumin and egg albumin were studied), which gel only at higher protein (2%) and high formaldehyde concentration (10%), in a narrow pH zone around the isoelectric point. The mechanism of gel formation was studied in more detail with fibrinogen. Samples treated with formaldehyde under various conditions were compared with the native protein. There was no difference, as compared with the native protein, in the number of tyrosine or tryptophane groups, whereas a marked decrease (20%) occurred in the number of lysine ϵ-amino groups in those samples, and only those, which formed a gel. Using C_{14} formaldehyde it was

possible to show that the number of formaldehyde molecules found correspond to the decrease in the number of lysine ε-amino groups. The formaldehyde forms, thus, methylene bridges between the ε-amino groups and some unidentified groups of adjoining molecules. A slight decrease of the number of amide groups in the gelified samples suggests that, partly at least, amide groups may form the second leg of the bridge.

Significance to Heart Research: No direct significance.

Proposed Course of Project: Project was completed about June 1, 1958.

Part B included - No.

Serial No. NHI-143
1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Hyperlipemia of Experimental Nephrosis: Some Investigations into Pathogenesis. (Project started Aug. 1, 1958 - not completed.)

Principal Investigator: J. C. Allen

Other Investigators: J. H. Baxter, H. C. Goodman

Cooperating Units: None

Man Years (calendar year 1958)
Total: 0.25
Professional: 0.25
Other: 0

Patient Days (calendar year 1958)
None

Project Description:

Objectives: The hyperlipemia of human nephrosis is reversed by the administration of albumin as shown in this laboratory. It was hence decided to study the effects of altering various conditions, such as diet, serum osmotic pressure and blood coagulability, on experimental nephrotic hyperlipemia. The results of such experiments may help elucidate the pathogenesis of nephrotic hyperlipemia, as well as explain the response in patients as mentioned above.

Methods Employed: Nephrosis is induced in rats by the injection of rabbit anti-rat-kidney serum in a manner previously described from this laboratory. Methods for determining serum total lipids, total cholesterol and phospholipids have been adapted for micro-quantitative determinations in these animals. Using these methods serum lipids may be quantitated before and after the application of various experimental conditions.

Major Findings: The administration of dextran in hyperlipemic nephrotic rats causes return of all serum lipid values to normal levels. Short term administration of a carbohydrate polypeptide diet causes reduction of the high lipid values, which return to more abnormal levels on a low-fat balanced solid chow.

Serial No. NHI-143

Significance to Heart Research: The use of induced nephrotic hyperlipemia in experimental animals gives a useful investigative tool for study of this form of hyperlipemia. The results may be applicable to the general problems of hyperlipemia as well as to the analogous human disease of nephrosis.

Proposed Course of Project: The effects of various osmotically active substances (as PVP, gelatin, albumin), anticoagulants (as dextran sulfate, heparin) and dietary changes on this experimental hyperlipemia are to be studied.

Part B included: No.

Serial No. NHI-144
1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies on Soluble Protective Substance in Experimental Nephritis.
(Project started September, 1956 - not finished)

Principal Investigator: James H. Baxter

Other Investigators: Patricia Duffy, Howard Goodman, James Allen

Cooperating Units: None.

Man Years: (calendar year 1958)
Total: 0.4
Professional: 0.1
Other: 0.3

Patient Days: (calendar year 1958)
None.

Project Description:

Objectives: To study and purify the soluble substance derived from nephrotoxic antigen of rat tissue, which will neutralize antibodies nephrotoxic to the rat.

Methods Employed: The soluble protective substance is present in the supernatant solution prepared by digestion of rat kidney or lung with trypsin.

Major Findings: The preparation obtained by digestion of acetone powder of kidney is more active and contains less inert material after dialysis than obtained by digestion of kidney homogenate. The number of protective units per mg. of "protein" has been used as the index of purity at various stages. It is evident from studies in the analytical ultracentrifuge that much of the material determined as "protein" (after dialysis) is of relatively small molecular size. No considerable degree of purification of the protective substance has been accomplished by zone electrophoresis, chromatography on modified cellulose or preparative ultracentrifugation. One of the problems is inactivation of the protective material during manipulations.

Significance to Heart Research: The tissue antigen being studied may be important in the pathogenesis of nephritis and nephrosis.

Proposed Course: If the protective activity really belongs to a single molecular species - and this may not be the case - it should be possible to accomplish a considerable degree of purification by ultracentrifugation. A further attempt at purification by ultracentrifugation is planned.

Part B included: No.

Serial No. NHI-145
1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Proteolytic Fragmentation of the Myosin Molecule
(Project started June 1, 1958 - completed Oct. 15, 1958)

Principal Investigator: Elemer Mihalyi

Other Investigators: William F. Harrington, M. I. Knoller

Cooperating Units: Section of Cellular Physiology

Man Years (calendar year 1958)
Total: 1.5
Professional: 1.0
Other: 0.5

Patient Days (calendar year 1958)
None

Project Description:

Objectives: Myosin is a very large molecule, much too large to be attacked by structural investigations in its intact form. It was thought that the proteolytic fragmentation of the myosin, since it yields well defined fragments, may be used advantageously in structural investigations, especially to establish connections between the different functions and certain localized areas of the molecule and, on the other hand, the proteolytic process itself may shed some light on the structure of the molecule.

Methods Employed: Viscosity, optical rotation, sedimentation, pH-stat titration.

Major Findings: The fragmentation of the myosin molecule was followed in the ultracentrifuge and at the same time the number of peptide bonds split was also determined by physicochemical methods and also confirmed by chemical analysis. It appears that some of the peptide bonds are split at a rate about ten times faster than that of the remainder. This fast reaction accounts for the formation of the large fragments. Some optical evidence suggests that parts of the myosin molecule are formed of a tightly coiled chain, whereas others are much looser and randomly folded. Apparently the enzyme attacks with much greater ease the random portions and thus liberates the tightly coiled fragments, giving the erroneous impression of liberation of preexistent subunits. This reaction

may prove of more general use. There are indications that collagen and fibrinogen are degraded by proteolytic enzymes in much the same way. Thus, at least with fibrous proteins, it is possible that the proteolytic breakdown of the molecules, under suitable conditions, can be used to estimate the proportion of the molecule in the tightly coiled form as opposed to the random portion. So far, only optical methods have been used for this purpose.

Significance to Heart Research: Myosin is undoubtedly the most important of the muscle proteins. Muscular contraction is brought about by some yet unknown configurational change of the myosin molecule and the energy required by this process is liberated also by the myosin molecule, through its enzymatic function directed toward the breaking of a high energy phosphate bond. Thus, the myosin molecule seems to be a complete unit to convert chemical energy into mechanical work. Knowing its complete structure is, therefore, a prerequisite to the understanding of muscular contraction.

Proposed Course of Project: All the experimental work has been completed. Manuscript for publication will be ready in a few weeks.

Part B included: No.

Serial No. NHI-146
1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Development of a Method for Counting Water Soluble Compounds in the Liquid Scintillation Counter. (Started April, 1958 - not completed)

Principal Investigator: Daniel Steinberg

Other Investigator: Ray Pittman

Cooperating Units: None

Man Years (calendar year 1958)
Total: .2
Professional: .1
Other: .1

Patient Days (calendar year 1958)
None

Project Description:

Objectives: To make it possible for the biochemist to obtain radioassay on polar compounds directly without the need of first converting them to an organic soluble form. Presently available methods for dealing with water soluble compounds are either limited to small quantities or involve time consuming conversion procedures.

Methods Employed: The basic principle is the use of a two-phase system - a solid phase consisting of finely divided fluorescent material and a liquid phase containing the compound to be assayed. The earlier studies utilized a plastic with diphenylstilbene in it (pilot B); later it was found that crystals of anthracene and of diphenyloxazole were also suitable for the solid phase.

Major Findings: By reducing the ratio of fluid volume to solid surface it has been possible to shorten the mean path for the beta particles of C^{14} prior to their collision with the fluorescent material. With the plastic scintillator maximum efficiency was obtained using tightly packed filaments and adding the aqueous or alcoholic solution into the fine interstices. Efficiencies up to 29% were obtained. Using a large number of short segments or beads efficiencies of 10 to 12% were obtained. The sample can be recovered unchanged, which is a distinct advantage in some studies.

Significance to Heart Research: These studies represent a basic improvement in radioassay and the technique should be useful in many kinds of research, including research in the area of heart disease.

Proposed Course of Project: Attempts are being made to assess the reproducibility of anthracene, which would be cheaper than the plastic materials and more easily available.

Part B included Yes.

Serial No. NHI-146

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Steinberg, D. Radioassay of C^{14} in Aqueous solutions using a liquid scintillation spectrometer. Nature 182: 740-741, 1958.

Steinberg, D. Radioassay of C^{14} and tritium in aqueous solutions in the liquid scintillation spectrometer. In Proceedings of the Symposium on Tritium in Tracer Applications. New York City, Oct. 31, 1958. In Press.

Honors and Awards relating to this project:

None.

Serial No. NHI-147
1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Establishment of an In Vitro System for Fat Absorption. (Project started March 1, 1958 - not completed)

Principal Investigator: Alan F. Hofmann

Other Investigators: None; this work has been done with the supervision of Dr. Joseph Bragdon.

Cooperating Units: None

Man Years (calendar year 1958)
Total: .33
Professional: .33
Other: 0

Patient Days (calendar year 1958)
None

Project Description:

Objectives: The purpose of constructing an in vitro system for fat absorption is two-fold: First, the demonstration of incorporation of labeled amino acids into chylomicron protein, if hepatic lipoprotein synthesis and exchange can be excluded, would be suggestive evidence for protein synthesis by intestinal mucosa cells. Second, an in vitro system of fat absorption would permit further study and possibly quantitation of the lipid species which are being absorbed.

Methods Employed: The apparatus consists of a simple air lift pump for the perfusion of a rat's intestinal loop, either with or without an intact blood supply. If the blood supply is excluded, oxygen for cellular metabolic processes is supplied by saturating the luminal perfusing fluid with oxygen. To date, histochemical and optical density methods for gauging fat absorption have proven inadequate, and in the future, isotopic fatty acids are to be used, with counting of the mucosa and chyle, the latter obtained by thoracic duct cannulation. The perfusing fluid may be sampled at any time for lipid or isotopic analysis.

Major Findings: The isolated perfused loop suspended in buffer does not release chylomicra into the bath when perfused with a fatty mixture, which in theory whould be optimal for fat absorption. The same mixture, when perfused through a gut with blood supply intact in the anesthetized rat, shows a significant fall in total

lipid content, but produces minimal chyle, suggestive of mucosal absorption, but reduction in chylomicron synthesis. The effect of ether anesthesia in reducing chyle production is well known, but has not been studied in detail.

Significance to Heart Research: This research is intended to define in more detail the chemistry of chylomicra, the large lipoprotein by which fat passes from the intestinal mucosa into the blood stream. Patients who cannot remove chylomicra from the blood stream are known to have an increased incidence of atherosclerosis, and atherosclerotic lesions are a major cause of myocardial disease.

Proposed Course of Research: We have succeeded in outlining the major difficulties in the construction of such a system for fat absorption, and should be able in subsequent months to succeed in getting at least some fat absorption under the proposed conditions. The mechanisms by which anesthesia inhibits fat absorption may be of physiologic significance, and using thoracic duct cannulated animals, we hope to study this in more detail, specifically, whether factors which are known to be released during ether anesthesia, e.g. epinephrine stimulate fat absorption, and whether the alteration of intestinal motility by intravenous serotin can counteract the effects of ether anesthesia. These studies are to be pursued after the completion of the initial project, namely the demonstration of chylomicron protein synthesis by the intestinal mucosa cell.

Part B included: No.

Serial No. NHI-148
1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: In vitro Incorporation of Sterols into Serum Lipoproteins
(Project started June, 1958 - completed September, 1958)

Principal Investigator: Joel Avigan

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958)
Total: 0.3
Professional: 0.3
Other: None

Patient Days (calendar year 1958)
None

Project Description:

Objectives: To investigate the incorporation of sterols, notably cholesterol, into serum lipoproteins in vitro in order: (1) to find a method for efficient labeling of lipoproteins for metabolic studies, and (2) to learn about the affinity of the lipoprotein complex for cholesterol.

Methods Employed: A pentane solution of isotopically labeled sterol was added to Celite 545; the solvent evaporated and the treated Celite incubated at 37° for 2-24 hours with human or animal serum or with an isolated serum lipoprotein. Subsequently, the solution was passed through an ultrafilter. The preparations thus obtained were analyzed for radioactivity and in some experiments administered intravenously to humans or animals for metabolic studies.

Major Findings: Cholesterol and several other sterols, when dispersed on Celite, dissolve in whole human or rat serum or in isolated serum lipoproteins. Most of the cholesterol dissolved in serum is associated with various lipoprotein fractions. Cholesterol incorporated into serum lipoproteins by the present method behaves, when administered intravenously, like cholesterol incorporated biosynthetically with respect to its disappearance rate from serum. On the other hand, labeled cholesterol in the form of suspension rapidly disappears from the circulation into the various organs and then reappears in the serum.

Significance to Heart Research: (1) The method described enables one to incorporate into serum much larger concentrations of labeled cholesterol than those which can be readily achieved by feeding or injecting labeled cholesterol. Consequently, the procedure may be of great importance for metabolic studies particularly when the fate and distribution of circulatory cholesterol has to be followed for prolonged periods. (2) The phenomena observed suggest that also *in vivo* the removal of cholesterol from the cells and its transport in serum may take place by binding with a preexisting lipoprotein complex.

Proposed Course of Project: The project has been completed and the results submitted for publication. An analogous study designed to incorporate triglycerides into lipoproteins is being contemplated.

Part B included: No.

Serial No. NHI-149

1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies on the Biosynthesis of Serum Lipoproteins (Project started 7/1/57-not completed)

Principal Investigators: Charles M. Radding, Daniel Steinberg, Joseph H. Bragdon

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958)

Total: 1.5
Professional: 1.5
Other: None

Patient Days (calendar year 1958)

None

Project Description:

Objectives: To determine the site of synthesis of the serum lipoproteins and to study the factors regulating synthesis.

Methods Employed: Using C^{14} labeled amino acids we have been studying the synthesis of lipoproteins by rat liver slices and by the perfused rat liver. Lipoproteins have been isolated by the usual ultracentrifugal techniques and the identity of the isotopically labeled lipoproteins with serum lipoproteins has been studied by a method involving paper chromatography high voltage paper electrophoresis, and autoradiography.

Major Findings: 1) Both liver slices and the perfused rat liver incorporate labeled amino acids into serum lipoproteins. 2) In the liver slice system the labeling of lipoproteins has been shown to have attributes of genuine protein synthesis: incorporation of labeled amino acids is energy dependent and is inhibited by p-fluorophenylalanine, an amino acid analogue known to inhibit protein synthesis in other systems. 3) Preliminary evidence from autoradiography of chromatograms indicates that the labeled lipoproteins are in fact identical with or extremely similar to the serum lipoproteins.

PHS-NIH
Individual Project Report
Calendar Year 1958

Significance to Heart Research: The *in vitro* system devised with rat liver slices should provide a powerful tool for studying the synthesis of serum lipoproteins. From such a study we may learn about some of the physiological controls of blood lipid levels. Knowledge of such controls is of prime interest in understanding the pathogenesis and possible prevention of atherosclerosis.

Proposed Course of Research: 1) Studies designed to identify the synthesized lipoproteins with serum lipoproteins are nearly completed. 2) Studies are in progress on the physiological factors affecting lipoprotein synthesis. We propose also to attempt to work with cell-free systems in order to study the more basic aspects of the linkage of protein and lipid into lipoprotein.

Part B. Included - Yes.

PHS NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Radding, C. M., Bragdon, J. H., and Steinberg, D. The synthesis of low- and high-density lipoproteins by rat liver *in vitro*, Biochim. et Biophys. Acta. 30: 443-444, 1958.

Honors and Awards relating to this project: None.

Serial No. NHI-150

1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Treatment of the Nephrotic Syndrome
(Project started 1954- not completed)

Principal Investigator: Howard Goodman

Other Investigators: James Baxter, James Allen, Robert Bowser (tech.)

Cooperating Units: None

Man Years (calendar year 1958)

Total: 1.25
Professional: 1.0
Other: 0.25

Patient Days (calendar year 1958)

600

Project Description:

Objectives: Studies of the effect of adrenal corticoid therapy on patients with the nephrotic syndrome have been continued with the aim of (1) learning more about the pathogenesis of the renal lesion, (2) learning more about the mechanism of the anti-inflammatory action of adrenal steroids, and (3) determining which types of renal lesion are susceptible to suppression by the steroids.

Methods Employed: Screening of patients suitable for admission as well as follow-up studies on discharged patients was done in the weekly renal clinic in the outpatient department. Suitable patients were hospitalized, and after studies of lipid and protein metabolism, percutaneous renal biopsy was performed and a three week course of 40 mg prednisone daily instituted. Suitable blood and urine chemistry values were determined.

PHS-NIH
Individual Project Report
Calendar Year 1958

Patient Material, Major Findings: Since these studies were initiated in 1954, 45 patients with the nephrotic syndrome have been studied, as well as many other patients with proteinuria without the other characteristics of the nephrotic syndrome. Results of treatment are in the first 37 patients essentially as those reported in the publication listed below for the first 20 patients.

	Number	Complete remission or " " + Slight residual proteinuria	Partial Remission	No Remission
Children	(23)	16	3	4
Adults	(14)	7	5	2

Preliminary assessment of renal biopsy results indicate that no specific type of glomerular pathology found so far can be used to predict response to steroid therapy. Both thickened basement membranes and cellular proliferation have been seen in patients whose renal lesion could be suppressed by steroids.

Significance to Heart Research: The value of critical evaluation of the use of steroid therapy in patients with nephrotic syndrome is self-evident. Correlation of these clinical studies with the results of histological studies of renal biopsies in these patients may help both to clarify our concepts of the natural history of renal disease, as well as to characterize better the type of glomerular pathology susceptible to suppression by adrenal steroids.

Proposed Course of Project: These studies will be continued. An increasing percentage of patients to be admitted are those "resistant" to steroid therapy to determine if this is a quantitative or qualitative resistance by the use of higher dosage of corticoids than those used heretofore. It is hoped that electron microscope studies of biopsy specimens can be instituted in the Pathology Department.

Part B. Included - Yes

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Goodman, H. C. and Baxter, J. H. Adrenocorticotrophin and corticoid treatment of the nephrotic syndrome. Metabolism 7: 40-51, 1958.

Honors and Awards relating to this project:

None.

1. Laboratory of Cellular Phys. and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies on the Mechanism of Protein Synthesis
(Project started August, 1956 - Not completed)

Principal Investigators: Martha Vaughan, Daniel Steinberg

Other Investigators: Walter Lewis (technician)
Dr. Boyd O'Dell (guest worker for 3 months)

Cooperating Units: None

Man Years (calendar year 1958)
Total: 3.2
Professional: 2.2
Other: 1.

Patient Days (calendar year 1958)
None

Project Description:

Objectives: To study the incorporation of certain unnatural amino acids into proteins as a means of learning something about the specificity of the biosynthetic mechanism and to examine tissue extracts for the presence of compounds which might be intermediates in protein biosynthesis.

Methods Employed: In vitro incubation of tissues. Fractionation, separation and purification of proteins, peptides and amino acids using solvent and salt fractionations, protein crystallization, column and paper chromatography, electrophoresis. Quantification of these materials using chemical and spectrophotometric methods. Radioassay of H^3 and C^{14}.

Major Findings: Last year it was found that H^3-p-F-phenylalanine (and also H^3-o-F-phenylalanine) could be incorporated into lysozyme and into ovalbumin as well as into a mixed protein fraction by mixed hen's oviduct. It has been established that the analogue is bound in peptide linkage and is distributed among several peptides isolated from each of these purified proteins. The ability of liver slices to incorporate several other amino acid analogues into total TCA precipitable protein has been investigated. Ortho-tyrosine is incorporated into liver protein to roughly the same extent as are the fluoro-phenylalanines. Meta-tyrosine and norleucine are also incorporated but to a considerably lesser extent. The in-

PHS-NIH
Individual Project Report
Calendar Year 1958

corporation of all of these amino acids is inhibited by dinitrophenol. The incorporation of norleucine (present at a concentration of ca. 1.5×10^{-3} M) is inhibited roughly 25% by 3×10^{-3} M leucine or methionine but not at all by 10^{-3} M. In similar studies it has not been possible to demonstrate incorporation of norvaline, o-methyl serine or β-2 thienylalanine.

A non protein (TCA soluble) fraction of liver from which free amino acids have been removed with an ion exchange resin has been examined in several studies. The incorporation of radioactivity from labelled amino acids (alanine, phenylalanine, lysine, leucine and the fluorophenylalanines) into this fraction by rat liver slices has been observed. Some of this radioactivity undoubtedly represents acidic degradation products of the labelled amino acid. But in the case of lysine, phenylalanine, p-fluorophenylalanine there is incorporation of radioactivity into this fraction which is recoverable as amino acid after acid hydrolysis. The formation of this "bound amino acid" is inhibited by dinitrophenol.

The nature of these radioactive compounds and their role in metabolism has not been elucidated. A batch of this material prepared from the liver of a rat which had received a mixture of C^{14} labelled amino acids prior to sacrifice was fractionated by chromatography on ECTEOLA. Several peaks of radioactivity were found, overlapping but in most cases not coinciding with peaks of 260 absorbing material. (It has been found that most of the nucleoside, mono-, di- and triphosphates can be separated on this column and much of the 260 absorbing material in this fraction likely represents known nucleotides). On further fractionation of certain samples the UV absorbing material was separated from the radioactivity and hydrolysis of these radioactive compounds yielded in each case several amino acids (most of them not radioactive).

Significance to Heart Research: This is a part of the basic research program of the Heart Institute.

Proposed Course of Project: The work with amino acid analogues are essentially completed except for some studies in progress which may indicate what amino acid (s) is replaced when the fluorophenylalanines are incorporated. Experiments in progress are designed to give a rather complete survey of the amino acid containing compounds in this liver preparation. Since heretofore the fractions examined (chosen essentially at random) have contained a surprisingly uniform group of amino acids, it will be of interest and possibly of assistance in determining the structure of these compounds and their metabolic role to know whether the compounds do in fact contain only a certain restricted group of amino acids or whether this early impression is due to inadequate sampling.

Part B. included - Yes

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Vaughan, Martha and Steinberg, D. Incorporation of amino acid analogues into crystalline Proteins. Symposium on proteins. Proceedings of the 4th International Congress Biochemistry, Vienna, September 1-6, 1958. Pergamon Press (In Press).

Steinberg, D., Vaughan, M., Anfinsen, C. B., Gorry, J. D., and Logan, J. The preparation of tritiated proteins by the Wilzbach method and a simple method for liquid scintillation counting of radioactive proteins. "Liquid Scintillation Counting" London, England, Pergamon Press, 1958.

Honors and Awards: None

Serial No. NHI-152

1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A.

Project Title: Studies on the Mechanism of Action of Dietary Fats in Relation to Serum Lipoproteins.
(Started July, 1957 - not completed)

Principal Investigators: Daniel Steinberg and Joel Avigan.

Other Investigators: Hugh Vroman

Cooperating Units: None

Man Years (calendar year 1958)		Patient Days (calendar year 1958)
Total:	1.5	1190
Professional:	0.9	
Other:	0.6	

Project Description:

Objectives: To determine the nature of the changes in lipoprotein and cholesterol metabolism effected by dietary fats and to explore the mechanisms of action involved.

Methods Employed: Methods used in animal studies are described in last year's annual report. Clinical studies have been carried out using liquid formula diets containing either coconut oil or an unsaturated vegetable oil contributing 60% of the total caloric intake. Patients received 4-C^{14}-labeled cholesterol intravenously, given in the form of a complex with the patient's own serum lipoproteins (see 1958 annual report of Dr. Avigan for method). Serum samples are taken at intervals for determination of cholesterol level and cholesterol specific radioactivity. Complete fecal collections are made and the excretion of radioactivity in the form of sterol and of bile acids is determined.

Patient Material, Major Findings: (1) The marked difference between saturated and unsaturated fats in their effect on serum cholesterol levels has been confirmed.

(2) It has been found that a diet containing less than 5 grams of fat effects almost as great a lowering of the serum cholesterol as does a diet rich in unsaturated fat. However, the response is more rapid with unsaturated fats and the absolute lowering is somewhat, although only slightly, greater.

(3) The vegetable sterol content does not contribute importantly to the cholesterol lowering action of safflower oil. A preparation with sterol content around 0.1% was as effective as untreated safflower oil.

(4) An unexpected finding, in apparent conflict with some conclusions in the literature, was the finding that a very large percentage of the radioactivity appearing in the feces was in the form of sterol. Previously it has been assumed that bile acid represented essentially the only end product of cholesterol metabolism. In six patients studied, the percentage excretion as cholesterol ranged from 35 to 80%. The remainder of the radioactivity appeared in the form of bile acids.

(5) Some patients showed an increased excretion of cholesterol and bile acids when placed on a diet rich in unsaturated fat but others, despite a significant response of serum cholesterol level, failed to show any increase in excretion of end products. It was concluded that the metabolic effect of unsaturated fats could not be simply one of increasing the rate of conversion of cholesterol to bile acids.

(6) The curve for serum cholesterol specific radioactivity showed no obvious change in slope coincident with change in dietary fat. A finding of great interest was a transient but definite elevation in specific radioactivity upon the addition of unsaturated fat to the diet in two patients. This observation can only be due to mobilization of cholesterol from a store of higher specific radioactivity.

(7) Post-mortem tissue samples from a patient dying of a stroke some three months after he had received radioactive cholesterol were obtained and the specific radioactivity of tissue cholesterol determined. The specific radioactivity of cholesterol in brain, kidney, lung and spleen was considerably higher than that of liver cholesterol. This "cross-over" in specific radioactivity between the serum-liver pool and peripheral pools has been now demonstrated in animal studies. These findings, together with the previously reported findings in rats, constitute evidence for an effect of

dietary fat on the distribution of cholesterol between the various body pools. The nature of the primary action of dietary fats remains unknown and the results of these studies suggest that it is more complex than a simple effect on the rates of cholesterol synthesis and degradation, although these are apparently altered in the course of the changes induced by dietary fats.

Significance to Heart Research

Since the use of unsaturated fats in the diet offers great promise as an approach to hypercholesterolemia an understanding of the mechanisms involved is of obvious importance to those interested in treatment and prevention of atherosclerosis.

Proposed Course of Project:

The findings with respect to the hitherto unsuspected role of cholesterol excretion as such calls for further studies of the origins of this fecal sterol. An attempt will be made to determine whether the liver cholesterol content increases in man in response to unsaturated dietary fats in a manner analogous to that previously reported for rats. Because of the difficulty in interpreting studies using labeled cholesterol (due to the ease with which isotopic exchange reactions occur) an extension of this study using labeled proteins in the lipoprotein fractions is under consideration.

PART B. included - Yes

 Serial No. NHI-152

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Avigan, J. and Steinberg, D. Effects of saturated and unsaturated fat on cholesterol metabolism in the rat. Proc. Soc. Exptl. Biol. and Med. 97: 814-16, 1958.

Honors and Awards: None

Serial No. NHI-153

1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Metabolism of Plasma Unesterified Fatty Acids (Started in 1955 - not yet completed)

Principal Investigator: Robert S. Gordon, Jr.

Other Investigator: Miss Amy Cherkes

Cooperating Units: None. This project is partly in common with project of Dr. Donald S. Fredrickson of this Section. Please see his report.

Man Years (calendar year 1958)
Total: 1.25
Professional: .25
Other: 1.

Patient Days (calendar year 1958)
50

Project Description:

Objectives, Methods Employed, Patient Material, Major Findings: Clinical studies on the turnover and metabolism of C^{14}-labeled unesterified fatty acids have been covered in the report by Dr. Donald S. Fredrickson.

In addition to these investigations, in vitro studies utilizing adipose tissue of experimental animals have demonstrated that this isolated tissue will produce unesterified fatty acids. The role of various hormonal and nutritional factors in controlling the output of esterified fatty acids is being investigated. In addition, it has been demonstrated that heparin will cause the liberation of lipoprotein lipase from adipose tissue. The relationship of this enzyme to the process of UFA release is being studied.

Significance to Heart Research: The study of the metabolism of lipids is felt to be of importance in the ultimate understanding of atherosclerosis.

Proposed Course of Project: Further experiments will be undertaken along the lines already indicated.

Part B. included - Yes

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B:

Publications: Gordon, R. S., Jr. and Cherkes, Amelia. Production of unesterified fatty acids from isolated rat adipose tissue incubated *in vitro*. Proc. Soc. Exper. Biol. and Med. 97: 150-51, 1958.

See also reports of Dr. Joseph H. Bragdon and Dr. D. S. Fredrickson for titles of cooperative publications.

Honors and Awards:

None

Serial No. NHI-154

1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A.

Project Title: Relation between Unesterified Fatty Acid Metabolism and Lipoprotein Formation and Transport. (Project started: February, 1957-not completed)

Principal Investigators: DeWitt Goodman and Daniel Steinberg

Other: Walter Lewis

Man Years (calendar year 1958)

Total: .7
Professional: .5
Other: .2

Patient Days (calendar year 1958)

None

Project Description:

Objectives: A number of fatty acid analogues were explored in the hope of finding a compound which would successfully compete with normal fatty acids for binding on serum albumin. With such a compound it is hoped that it will be possible to block the normal transport of unesterified fatty acids and then observe how the body solves the problem of fat transport by using alternative mechanisms, presumably lipoprotein synthesis and transport.

Methods Employed: 3, 3-dimethyl-13-phenylmyristic acid was synthesized, tritiated by the Wilzbach method, repurified by counter-current distribution and administered to rats.

Major Findings: It was shown that this compound is not metabolizable by the rat at any significant rate, recoveries being over 90% after 24 hours. In vitro studies with rat liver slices again showed little or no metabolism. Continuous intravenous infusion of the compound as the albumin complex failed to have any important effect on the rate of oxidation of simultaneously administered C^{14}-palmitic acid. No marked changes in serum lipid levels occurred.

PHS-NIH
Individual Project Report
Calendar Year 1958

(Major Findings, continued)

A finding of considerable interest is that a very high percentage of the administered material is still present in the form of unesterified fatty acid even 24 hours later, whereas little or no C^{14} palmitic acid was found in this form at a corresponding time. The adipose tissue contained significant amounts of the analogue in unesterified form. A small fraction was incorporated into triglyceride and phospholipid so that it is possible to conclude that it can be handled by the tissue enzymes but at a considerably reduced rate.

Significance to Heart Research: These studies are a part of the continuing program in this section designed to clarify the mechanisms determining serum lipoprotein levels, which in turn may lead to an understanding of hypercholesterolemia.

Proposed Course of Project: In order to test the original hypothesis a larger amount of material will be necessary than was available at the time these studies were carried out. This material is now being synthesized on a contract and it will be administered in larger amounts to rats as soon as it becomes available.

Part B. Included - Yes.

PHS NIH
Individual Project Report
Calendar Year 1958

PART B: Honors, Awards and Publications

Publications other than Abstracts from this project:

Goodman, D. S. and Steinberg, D. Studies on the metabolism of 3, 3-dimethyl phenylmyristic acid, a non-oxidizable fatty acid analogue. J. Biol. Chem. 233:1066-71, Nov. 1958.

Honors and Awards relating to this project: None.

Serial No. NHI-155

1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A.

Project Title: Pathogenesis of Artherosclerosis
(Project started 1948 - not completed)

Principal Investigator: Joseph H. Bragdon

Other Investigators: Alexander Michajlik (Rockefeller fellowship from Warsaw since September, 1958)
Carlos Schultz
Carl Lauter and Edward Mougey (these technicians, although under my supervision, spend most of their time running a general service laboratory in lipid chemistry).

Cooperating Units: None

Man Years (calendar year 1958)

Total: 2.33
Professional: 1.33
Other: 1

Patient Days (calendar year 1958)

None

Project Description:

Objectives: To study the pathogenesis of atherosclerosis through normal and abnormal lipid metabolism.

Methods Employed: Serum lipoproteins are fractionated into several classes in the preparative ultracentrifuge. Fractions are quantitatively recovered and chemically analyzed. In some cases the lipoproteins are labelled isotopically.

Major Findings: Rat livers have been perfused with whole rat blood, to which C^{14}-labelled amino acids have been added. It has been found that the liver synthesizes beta lipoproteins at a rate twice that of alpha lipoproteins, which is synthesized in turn at a rate twice that of the residual serum proteins.

PHS-NIH
Individual Project Report
Calendar Year 1958

(Major Findings continued:) The search continued for a laboratory animal that will respond to the feeding of saturated and unsaturated fats in a manner similar to man. The mongolian gerbil was found not to. A group of squirrel monkeys are now available.

It was discovered that the feeding of corn oil to rabbits previously made atherosclerotic increases the rate of regression of the lesions when compared to similar animals fed coconut oil. This is the first time a substance has been found which increases the rate of regression of lesions. These experiments are being repeated with more control material to increase the statistical significance of the results.

Experiments from this laboratory in both rat and dog have shown that fat absorbed in the form of chylomicrons goes directly to the tissues. Gofman and the Donner group have claimed, without published evidence, that in man they are degraded in the blood stream, into lipoproteins of increasing density. Experiments are underway to confirm or deny this phenomenon in man.

Significance to Heart Research: Atherosclerosis causes coronary heart disease, which is the leading cause of death.

Proposed Course of Project: To continue along same lines.

Part B Included - Yes.

PHS-NIH
Individual Project Report
Calendar Year 1958

PART B. Honors, Awards and Publications

Publications other than abstracts from this project:

1. Bragdon, J. H. Lipoprotein Lipase. In a monograph edited by F. Homburger, S. Karger, New York, 1958.

2. Bragdon, J. H. and Gordon, R. S., Jr. Tissue distribution of C^{14} after the intravenous injection of labelled chylomicrons and unesterified fatty acids in the rat. J. Clin. Invest. 37: 574-578, 1958.

3. Bragdon, J. H. Current Trends in Atherosclerosis Research. A.M.A. Arch. Ind. Health 18: 222-227, 1958.

4. Bragdon, J. H. $C^{14}O_2$ excretion after the intravenous administration of labelled chylomicrons in the rat. Arch. Biochem. Biophys. 75: 528-533, 1958.

5. Bragdon, J. H. On the composition of chyle chylomicrons J. Lab. and Clin. Med. 52: 564-570, 1958.

Honors and Awards relating to this project:

None

Serial No. NHI-156

1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A.

Project Title: Studies of Hyperlipidemic States in Humans (Project started 1956 - not completed)

Principal Investigator: Donald S. Fredrickson (Studies on Hypocholesterolemic agents in collaboration with Dr. Steinberg (cf. his report for detailed coverage of project).

Other Investigators: Dr. Duncan McCollester (until July1, 1958)
Mr. Katsuto Ono, technician

Cooperating Units: None

Man Years (calendar year 1958)

Total: 0.2
Professional: 0.1
Other: 0.1

Patient Days (calendar year 1958)

500 (including out-patient hours)

Project Description:

Objectives: The observation and study of patients with abnormal concentrations of the various blood lipids, with particular emphasis on patients with so-called essential (familial) hyperlipidemia. Data is accumulated bearing especially upon classification, progress and insight into mechanisms of these diseases and to means of treatment.

Methods Employed: A weekly outpatient clinic is maintained at which patients referred because of significant hyperlipidemia are seen. Total lipid analyses, and rarely, lipoprotein analyses are obtained. Appropriate patients are selected for continuing follow-up and a number become available for both out- and in-patient studies related to lipid metabolism and hypocholesterolemic agents. Approximately 50 area patients with hyperlipidemias are available for certain of these studies.

PHS-NIH
Individual Project Report
Calendar Year 1958

Patient Material and Major Findings:

1) Of several drugs investigated as potential hypocholesterolemic agents, one, Benzmalecene (Merck, N-Cl-methyl-2, 3-di-p-chlorophenylpropylmaleamic acid) has been found to lower cholesterol levels in two of three patients. In the third, transient hyperlipemia developed. Side effects include elevated BSP retention and gastric distress. No conclusions can be made yet as concerning its clinical value. For animal studies, see Dr. Steinberg's report.

2) Sublingual heparin (Clarin, Leeming) has been tried in two hyperlipemic patients. No plasma lipoprotein lipase activity was observed after administration.

3) High amounts of unsaturated oils have been found to not elevate plasma cholesterol above values obtained on low animal fat diets; and within the limits of this form of therapy, encouraging results have been obtained with practical, palatable diets in one group of patients.

4) A number of interesting cases have been seen. One, a patient with the Hand-Schuller-Christian type of cholesterolosis, was found to have a large macroglobulin component in his serum proteins. He will be returned for further studies. Several patients with initially severe "essential" hyperlipemia (fasting triglycerides in one case 80 times normal) have been kept in almost complete remission for 12-24 months by dietary therapy alone.

Significance to Heart Research: Most of the patients in the category under study have a significantly higher incidence of coronary artery disease and represent an area of major concern in heart research.

Proposed Course of Project: To continue as above, with as many studies devoted to mechanisms producing these diseases as time and ideas permit.

Part B Included - Yes.

PHS-NIH
Individual Project Report
Calendar Year 1958

PART B. Honors, Awards and Publications

Publications other than Abstracts relating to this project:

Fredrickson, D. S., Peterson, R. E. and Steinberg, D., Inhibition of adrenocortical steroid secretion by Δ^4-cholestenone: Science, 127: 704-705, 1958.

Steinberg, D., Fredrickson, D. S., and Avigan, J. Effects of Δ^4-cholestenone in animals and in man, Proc. Soc. Exper. Biol. and Med. 97: 784-90, 1958.

Fredrickson, D. S. Atherosclerosis, Chapter 8. Metabolic Disturbances in Clinical Medicine, G. A. Smart, Editor, London, J. and A. Churchill, Ltd., 1958.

Fredrickson, D. S., Gordon, R. S., Jr., and Orloff, J. Cardiovascular Aspects of Metabolic Disease, Chapter 6, Ibid.

Fredrickson, D. S. Current Attitudes about Atherosclerosis, GP, 18: 102-106, 1958.

Honors and Awards relating to this project: None

Serial No. NHI-157

1. Laboratory of Cellular Physiology and Metabolism
2. Section on Metabolism
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A.

Project Title: Lipoprotein Metabolism in Nephrosis
(Project started September, 1955)

Principal Investigators: James H. Baxter and Howard Goodman
see separate report by James Allen

Other Investigator: Patricia Duffy. Some of the studies done in collaboration with Eleazar Shafrir and Daniel Steinberg.

Cooperating Units: None

Man Years (calendar year 1958)

Total: 1.4
Professional: 0.8
Other: 0.6

Patients days (calendar year 1958)

150

Project Description:

Objectives: a) to characterize the serum lipoprotein abnormalities in nephrosis, and b) to study some of the factors which influence the lipoproteins in nephrosis.

Methods Employed: Serum lipids and lipoprotein fractions separated by ultracentrifugation at various densities have been studied in 30 patients with nephrosis. In some of these cases the effects of prolonged glucose infusions, steroid therapy, or repeated infusions of serum albumin have been noted.

Patient Material, Major Findings: a) Sera from nephrotic patients with elevated lipids may be divided into 3 groups: group 1 - lactescent sera with elevation of the triglycerides and of the lipoproteins of D < 1.019, group 2 - slightly lactescent sera with elevation of lipoproteins of D < 1.019 and D 1.019-1.063, and group 3 - clear sera characterized chiefly by elevation of cholesterol and of lipoproteins of D 1.019 1.063.

PHS-NIH
Individual Project Report
Calendar Year 1958

b) Infusions of 10% glucose for 48 hrs. caused some patients with type 3 (clear) sera to change to the type 1 pattern. This did not occur in all patients, and it did not occur in normal controls. Unesterified fatty acid concentration was depressed during the infusions. In one patient who received C^{14} labeled glucose, the lipoprotein labeling was principally in the D < 1.006 and D 1.019-1.063 fractions at 6 hrs. If the label was largely limited to triglyceride the specific activity was greater in the D 1.019-1.063 fraction.

In several cases with type 1 sera, the pattern changed to type 3 early in remissions induced by steroid therapy. This change appeared to occur in at least one case before any considerable increase in serum albumin. The lipoproteins became normal.

In 2 cases with type 1 (lactescent) sera, repeated infusions of albumin brought about changes similar to those occurring early in remissions with steroid therapy, that is, there was a decrease in lipoproteins of D < 1.019 and an increase in those of D 1.019-1.063, and the sera became clear. In addition to this effect, albumin caused a decrease in cholesterol and phospholipid, and in TC/PL ratio, in most cases with either lactescent or clear serum.

The studies suggest that the low serum albumin level in nephrosis is one of the causes of the lipoprotein disturbance. It is not clear how or to what extent this factor operates. There undoubtedly are other factors which are important.

Significance to Heart Research: Abnormal lipoprotein metabolism not only occurs in nephrosis but is thought to play an important role in the development of atherosclerosis.

Proposed Course of Project: It is planned to continue these studies in rats or dogs made nephrotic by injections of nephrotoxic serum.

Part B. No.

Form No. OGP-2
Oct. 1957

PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
Laboratory of Chemistry of Natural Products

Estimated Obligations for FY 1959

Total:	$350,392
Direct:	$266,000
Reimbursements:	$ 84,392

Serial No. NHI-158
1. Laboratory of Chemistry
of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Ormosia Alkaloids

Principal Investigator: H. A. Lloyd, Ph.D.

Other Investigator: Antoinette Velasquez (technical)

Cooperating Units: None

Man Years
Total .75
Professional: .50
Other: .25

Patient Days
None.

Project Description:

Progress during Past Year

Work on the Ormosia alkaloids ormosanine, ormosinine, panamine and methylcytisine was carried through to the publication stage.

This work was undertaken because of the potent hypotensive activity observed for oxypanamine. A related compound, of the same order and kind of activity, was prepared from N-methylpanamine. These substances are oxidation products of complex structure; in the case of oxypanamine the parent compound has the empirical formula $C_{20}H_{33}N_3$. No further work, beyond that described in last year's report and in the paper, was initiated on the N_3 series of alkaloids.

A second series of compounds, with only two nitrogen atoms, was brought under detailed study. These were found to have the empirical formula $C_{14}H_{20-22}ON_2$. The major compound had lactam and secondary amine groups, and terminal methylene unsaturation. The side chain, carrying the methylene end group, was degraded stepwise. It was found to be a four-carbon chain. The two nitrogen-containing rings had properties resembling those of the sparteine (lupin) alkaloids.

Direction of Current Research

This work was terminated in July.

Part B included: Yes.

Serial No. NHI-158

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B: Publications

Lloyd, H. A. and Horning, E. C. Alkaloids of *Ormosia panamensis* Benth. and Related Species, J. Am. Chem. Soc., 80, 1506-1510 (1958).

Lloyd, H. A. and Horning, E. C. Isolation of N-Methylcytisine from *Ormosia stipitata* Schery. J. Org. Chem., 23, 1074 (1958).

Serial No. NHI-159

1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A. Amine Oxide Rearrangements.

B. Organic Bases of Human Origin

C. Base Methylation Studies.

D. Plant Organic Bases.

Principal Investigator: M. S. Fish, Ph.D.

Other Investigator: N. M. Johnson (technical)
E. P. Lawrence (technical)

Cooperating Units: None

Man Years
Total: 1.50
Professional: .50
Other: 1

Patient Days

None.

Project Description:

Progress during the Past Year

A. - Amine Oxide Rearrangements: A study of the rearrangement of N,N-dimethyltryptophan oxide under biological conditions was continued. The earlier chemical work demonstrated that a rearrangement reaction leading to N-demethylation occurred readily with ferric ion (in a co-ordination complex) as a catalyst, and the evidence was examined for the existence of this demethylation as an enzyme-catalyzed biological dimethylation reaction. Three effects were examined: (a) the effect of protein denaturation, (b) the added cofactor requirements and (c) comparative reactions with related compounds. The first effect was studied in the usual way by boiling the enzyme preparation; the system was inactive, and no reaction of any kind was observed. This is evidence supporting the enzyme-catalyzed nature of the reaction, which goes smoothly with a mouse liver homogenate. It is not conclusive evidence, however, since protein precipitation and denaturation might well be regarded as removing an active metal-bearing but non-enzymatic catalyst. The cofactor effects were studied in the usual way, and it was found that no added cofactors

were needed. Since all of the chemical evidence indicates a rather specific kind of catalytic action which does not involve oxidation when the oxide is used as a substrate, this result was not unexpected. Additional data was obtained by comparing the biological reactions of N,N-dimethyltryptamine oxide and N,N-dimethyltryptophan oxide. Under exactly the same conditions where a demethylation reaction was observed for the amino acid oxide, the dimethyltryptamine oxide did not undergo rearrangement-demethylation. Since it is known that the chemical (non-enzymatic) requirements for the reaction are the same in both cases, this is strong evidence that the observed reaction under biological circumstances was due to a biological (enzymatic) catalyst.

B. - Organic Bases of Human Origin: A procedure was developed for the isolation of organic bases (as a class) from human urine. The mixed bases were separated into two groups for study, indoles and a "phenolic" group. The indole class was investigated by the usual chromatographic and electrophoretic methods. Four substances were found. They were serotonin, tryptamine, dimethyltryptamine oxide and bufotenine. Serotonin has been recognized for some time as a human excretion product, and evidence relating to tryptamine was published while this work was in progress. The amounts of dimethyltryptamine oxide and bufotenine were quite low, and it was difficult to characterize these substances. The "phenolic" fraction was investigated separately. Seven compounds were found. None have been identified.

C. - Base Methylation Studies: Tryptamine was taken as an example of a human base that might be expected to undergo methylation. Two general routes of methylation are known as biological pathways (a) the S-adenosylmethionine route and (b) a sequence involving tetrahydrofolic acid and transfer of a one-carbon intermediate at the formaldehyde level. The second route is a reversal of the N-oxide demethylation pathway, and evidence was sought for the biological methylation of tryptamine by either of these routes. When the enzymatic transformation of tryptamine was examined, it was found that a neutral substance was formed, rather than another base. The new substance was characterized by chromatographic and electrophoretic means but its structure is not known. It is rather similar in its properties to N-formyltryptamine.

D. - Plant Organic Bases: A number of plants contain relatively simple organic bases derived from tryptophan, phenylalanine and tyrosine. Several of these were studied to obtain information about the occurrence of the bases and the relationships within each group. Prosopis juliflora.- This plant was found to contain tryptamine and phenylethylamine. The evidence for serotonin was positive but the data were not completed in a detailed way. It is of interest to note that the methylated and non-methylated compounds seem to occur in pairs - bufotenine and N,N-dimethyltryptamine, serotonin and tryptamine. Indole acetic acid was also found to be present. Piptadenia macrocarpa.- This plant contained an indole of an unknown structure (compound E) in addition to those already identified. A small quantity of "E" was isolated for study on a micro scale. It was characterized by chromatographic and electrophoretic means, along with its reduction product. Its structure is still unknown, but the data indicate that it is a 5-hydroxy structure with unsaturation in the side chain.

Piptadenia snuff.- This hallucinogenic material from Venezuela was examined for indole bases and it was found to contain bufotenine, bufotenine oxide, and four other indole bases. Acacia longifolia.- This was found to contain two indole bases. Abrus precatorius.- This was found to contain four tryptophan derivatives in addition to monomethyl tryptophan. Other plants.- A number of plants reported to contain hallucinogenic agents were examined. Most of the tests for organic bases were negative and no extended investigations were made.

Direction of Current Research

Work on these problems ended in July. Further work will be concerned with bringing some of the nearly completed projects to a terminal stage. The amine oxide work will be continued with emphasis on other aspects, including the mechanism of the rearrangement reaction. The enzymatic conversion of tryptamine is under study in another laboratory.

Part B included: No.

Serial No. NHI-160

1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: An Investigation into the Chemistry and Metabolism of the Glycolipides of Human Erythrocytes. (Project started July, 1958)

Principal Investigator: C. C. Sweeley, Ph.D.

Other Investigator: E. A. Moscatelli, Ph.D.

Cooperating Units: None.

Man Years
Total: .75
Professional: .75
Other:

Patient Days
None.

Project Description:

Progress during Past Year

Small quantities of complex lipide material were isolated from erythrocytes by Japanese workers. These lipides are composed of sphingosine, long-chain fatty acids, galactose, glucosamine and sometimes neuraminic acid. The family of glycolipides have not been further fractionated into pure compounds and this is the first objective of this investigation. Assay methods have been set up for phosphorus, nitrogen, reducing sugar and hexosamine.

A method for the isolation of glycolipides from human erythrocytes has been devised. The product by this solvent fractionation procedure is 30-40% pure. Preliminary results of partition chromatography on silicic acid indicate that pure compounds may be obtained by this procedure.

Direction of Current Research.

Chemical. - Further fractionation by chromatography will be studied in order to obtain pure samples of the glycolipides. Further assaying of spingosine and fatty acid will be made with a gas chromatography instrument.

Metabolism. - The biosynthesis of the glycolipides from simple precursors will be studied using bone marrow as the source of the enzyme system. The assay method will utilize C^{14}-labelled galactose.

Part B included: No.

Serial No. NHI-161

1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Enzymatic Studies on the N-Methylation of Amines.

Principal Investigator: C. C. Sweeley, Ph.D.

Other Investigator: M. S. Bronk (technical)

Cooperating Units: None

Man Years
Total: 1
Professional: .25
Other: .75

Patient Days
None.

Project Description:

Progress During Past Year.

Experiments were designed to study the N-methylation of histamine, tryptamine, tyramine and serotonin by tetrahydrofolic acid and formaldehyde or formate in the presence of pigeon liver enzymes.

An acetone powder of pigeon liver catalyzed the formation of N-acetylhistamine from histamine in the presence of coenzyme A. No formation of N-formyl or N-methyl amines could be demonstrated with any of the substrates tested.

Direction of Current Research.

No further studies are planned at the present time.

Part B included: No.

Serial No. NHI-162

1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Chemical and Enzymatic Transformations of Amine Oxides.

Principal Investigator: C. C. Sweeley, Ph.D.

Other Investigator: R. Levenberg (technical)

Cooperating Units: None.

Man Years
Total: 1
Professional: .25
Other: .75

Patient Days
None

Project Description:

Progress during the Past Year

Oxynicotine was prepared chemically by hydrogen peroxide oxidation. The product was isolated from paper chromatograms in pure form.

The microbiological oxidation of nicotine by a strain of Pseudomonas was studied in an attempt to implicate oxynicotine (one of the isomers) as an intermediate in the oxidative reaction. The Pseudomonas was grown aerobically on a synthetic medium containing nicotine as a source of carbon. Cell-free extracts were used directly or were further separated into soluble and particulate fractions. Particulate fractions were broken up by sonic oscillators and detergents. The enzyme fraction which degraded nicotine was found in the particulate fraction. Experiments in which nicotine was replaced by oxynicotine as a substrate were negative. The enzyme system was not active after sonic disintegration or detergent break-down of the particulate fraction.

Direction of Current Research

A comparison of the isomers of oxynicotine with the natural product from tobacco leaf will be made in order to establish the configuration of natural oxynicotine.

Part B included: No.

Serial No. NHI-163
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Isolation and Characterization of Components of the Callicrein System from Urine, Pancreas, and Blood Plasma.

Principal Investigator: Jack V. Pierce, Ph.D.

Other Investigator: Patricia A. Wagner (technical)
E. P. Lawrence (technical)

Cooperating Units: S. J. Sarnoff, L. C. Sarnoff, M. E. Webster, W. D. Fisher (Laboratory of Cardiovascular Physiology Serial No.)

Man Years
Total: 1.75
Professional: 1
Other: .75

Patient Days
None.

Project Description:

Progress during the Past Year

Introduction:

In our previous annual report, evidence was presented that the nondialyzable material in normal human urine responsible for vasodilatation and hypotension in the dog preparation of Sarnoff and coworkers is callicrein. This assumption is fully supported by further observations.

Werle and coworkers, in a series of papers starting in 1928 and continuing to the present,* have hypothecated the following: (1) Callicrein acts by proteolysis of an α_2-globulin, callidinogen, to release a

* See the summary of these up to 1950 in Frey, Kraut, and Werle, "Kallikrein (Padutin)", F. Enke Verlag, Stuttgart, 1950. Chapters I, II, III, IV, and VII are available in English translation at the NIH library.

slowly dialyzable peptide, callidin, which causes vasodilatation and hypotension in the dog and contraction of isolated guinea pig intestine. (2) Blood callicrein is normally bound to an inactivator protein and can act on callidinogen and thus produce its dilator effect by means of callidin only when the complex disassociates. (3) Blood contains an excess of callicrein inactivator. (4) Callidin is destroyed by a blood peptidase. (5) The callicreins from urine and pancreas may be identical, whereas these differ from salivary and serum callicreins which in turn are different from each other.

Because we are ultimately concerned with blood pressure regulation in man, particularly in its pathology, we are fractionating human starting materials for the isolation of the several components. Hog pancreatic callicrein is being used chiefly to explore untried methods.

Major effort is being placed on the isolation of pure callidinogen for the following reasons: (1) It should be possible to replace the present bioassays (guinea pig intestine and dog preparations) by in vitro enzymatic methods for the determination of callicrein, inactivators, callidin, and callidinogen. (2) Preparation of pure callidin would be greatly facilitated. (3) The question of the identity of callidinogen with other α_2-globulins yielding vasoactive peptides - such as hypertensinogen, bradykininogen, and pepsitensinogen - could be settled.

Experimental:

1. Callidinogen. The isolated guinea pig intestine assay for callidinogen and studies of the destruction of callidinogen by proteases present in human plasma are described in the current report of Dr. M. E. Webster.

Fractional ammonium sulfate precipitation of whole plasma (outdated human) has been tried many times under different conditions. Recovery has been poor, due in part to losses incurred during dialysis. Even when this effect was much reduced by the presence of added soybean trypsin inhibitor and when this indibitor was added at each fractionation step, complete recovery has not been realized and the activity occurs, in varying amounts, in all the precipitates (from 25 to 50 per cent of saturation).

Recent studies suggest the feasibility of using ion exchangers on a relatively large scale to yield a stable, purified preparation. Human plasma diluted five-fold with water and stirred at pH 4.0 with XE-64 gave about 75 per cent recovery of activity and 90 per cent recovery of non-dialyzable solids in the filtrate. The same diluted plasma, when stirred with DEAE* cellulose at pH 7.0 and 8.0, gave no activity in the filtrate and about 50 per cent in the phosphate buffer-sodium chloride eluate, accompanied by about 80 per cent of the starting nondialyzable solids. The non-dialyzable solids in the plasma were almost completely adsorbed by DEAE cellulose at pH 7.0 and 8.0 when the plasma was diluted twenty-fold.

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* Diethylaminoethyl

2. Callicrein. Previous work in this laboratory showed that human urinary callicrein (HUC) can be adsorbed by passing raw urine at pH 4.0 and at 2° C. over a column of XE-64 buffered at pH 4.0, and can be quantitatively recovered by elution with sodium phosphate buffer, pH 6.0. Conditions for the quantitative recovery of HUC from raw urine by batch operation have been worked out for XE-64. Urine was stirred with 10 grams of XE-64 (H^+) per liter, the pH was adjusted to 4.0 with hydrochloric acid, and stirring was continued for one hour at 18-20° C. The resin was recovered by filtration, washed with small portions of 0.2 M sodium acetate buffer, pH 4.0, stirred with sufficient sodium phosphate (about 0.1-0.2 M) to give a pH of 5.0 filtered, and washed on the filter with water. The filtrate, containing all of the starting callicrein activity, was dialyzed against running tap water at 20° C. for 24 hours. The dialysand was then frozen and lyophilized. The purification was 1,000-fold in total solids and 1-fold in nondialyzable solids. Such a preparation probably can be purified an additional 5- to 20-fold by gradient elution DEAE cellulose chromatography, since an earlier experiment showed that uranyl acetate precipitate from urine (25 per cent as pure as the XE-64 preparation) could be purified 15-fold when adsorbed on a DEAE column and eluted by stepwise change of eluents.

Preliminary attempts to obtain human pancreatic callicrein (HPC) have been made. Extraction of the minced pancreas with 0.05 N acetic acid and precipitation of the callicrein in the extract with uranyl acetate or lead acetate gave fairly good yields of HPC. Chromatography on DEAE cellulose and XE-64 should afford considerable purification: hog pancreas callicrein has been purified about 25-fold on an XE-64 column.

3. Callicrein Inactivators. Two callicrein inactivators (I and II) have been reported to be present in blood serum and have been distinguished by their ability to inactivate urinary and pancreatic, blood, and salivary callicreins and by their pH and temperature stabilities.

We have been able to adsorb an HUC inactivator from dialyzed outdated human plasma to DEAE cellulose and to elute it with 0.1 M sodium phosphate buffer, pH 7.0. All of the original inactivator activity was recovered in the eluate, though accompanied by about 65 per cent of the starting proteins. Presumably, gradient elution on a DEAE column would provide a greater purification.

Direction of Current Research

The results of plasma fractionation with XE-64 and DEAE cellulose to purify callidinogen will be thoroughly explored. Other methods also will be investigated.

Large scale recovery of callicrein from human urine by XE-64 adsorption will be undertaken, and the concentrate thus obtained will be purified further by chromatography and by other appropriate methods.

Methods for the purification of human pancreatic callicrein will be investigated.

Attempts will be made to devise a convenient method to obtain active human blood callicrein in theoretical or reproducible yields.

Work on the purification of human blood callicrein inactivators will be continued.

Part B included: Yes.

Part B. Publications.

Sarnoff, S. J., Case, R. B., Macruz, R., Sarnoff, L. C., Sussman, K. E. and Pierce, J. V. Observations on the Vasodilator Properties of Urine, Circulation Research, 6, 522- (1958).

Serial No. NHI-164

1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Structure of Amaryllidaceae Alkaloids.

Principal Investigator: W. C. Wildman, Ph.D.

Other Investigators: S. Uyeo, Ph.D.
Y. Inubushi, Ph.D.
Elizabeth A. Kielar (Technical)
Antoinette A. Velasquez (Technical)

Cooperating Units: None.

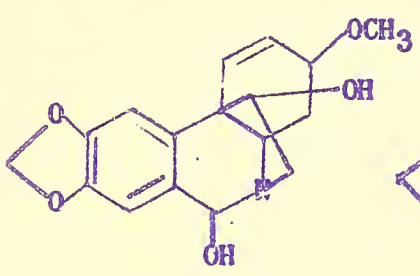
Man Years:
Total: 2.67
Professional: 1.67
Other: 1

Patient Days:
None

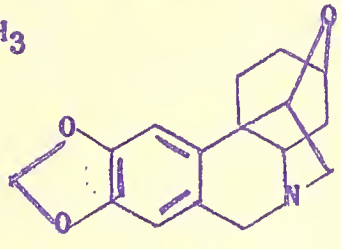
Project Description:

Progress during the Past Year

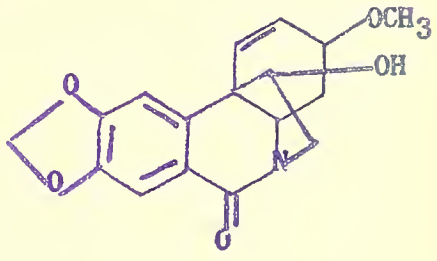
Conclusive chemical evidence has been obtained for the structure of haemanthidine (I). This represents a change from the report of last year in which an ethylene-imine type structure was considered. In agreement with structure (I) haemanthidine forms an O,O-diacetate. In warm acid (I) is converted to a demethoxy compound (II, R = OH). When (II, R=OH) was reduced catalytically and then treated successively with thionyl chloride and lithium aluminum hydride, a compound was obtained which was identical with dihydroapohaemanthamine (II). This conversion of haemanthidine established the nucleus of the alkaloid and located the



(I)



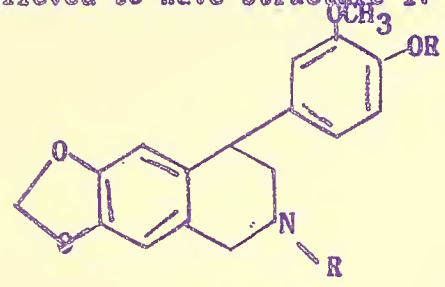
(II)



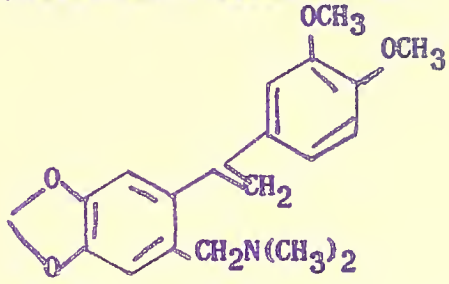
(III)

functional groups in rings C and D. Oxidation of haemanthidine with manganese dioxide give an oxocompound (III). Dihydrohaemanthidine, apohaemanthidine and dihydroapohaemanthidine gave analogous derivatives under the same conditions. These compounds are of immense interest since they, as predicted, exist and react as amino ketones, rather than lactams. Haemanthidine provides a connecting link between the spiro alkaloids (crinine, powelline, buphanidrine, etc.) and tazettine because haemanthidine methiodide is converted to tazettine by aqueous base and haemanthamine has been converted to (+)dihydrobuphanisine.

The alkaloids montanine and coccinine have been studied. These bases are isomeric and possess the same molecular formula, $C_{17}H_{19}NO_4$. They each contain one methylenedioxy, one methoxy and one hydroxyl group. Catalytic hydrogenation proceeds with the uptake of more than two moles of hydrogen. Apparently hydrogenolysis and hydrogenation occur together since the product contains a secondary amine. Oppenauer oxidation of either montanine or coccinine gives an optically active phenol which is believed to have structure IV (R=H). Methylation with diazomethane



IV



V

afforded a dimethyl derivative (IV, R = CH_3) which was degraded by the Hofmann method. The methine (V) was optically inactive and had the expected spectral properties. A dihydro derivative of V has been prepared and is in agreement with the expected physical and spectral properties.

Direction of Current Research:

The isolation and characterization of alkaloids and other physiologically active constituents of selected plant material will be continued. Preliminary studies on the biogenesis of selected alkaloids and the transformations of them by microbiological and mycological means will be started.

Part B included. Yes

Serial No. NHI-164

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B: Publications

Warnhoff, E. W. and Wildman, W. C., The Structure of Undulatine, Chemistry and Industry, 1293-1294 (1958).

Wildman, W. C., Alkaloids of the Amaryllidaceae. XI. The Structures of Alkaloids Derived from 5,10b-Ethanophenanthridine, J. Am. Chem. Soc., 80, 2567-2575 (1958).

Fales, H. M. and Wildman, W. C., Alkaloids of the Amaryllidaceae. XII. Interconversion of Alkaloids by Sodium and Amyl Alcohol, J. Am. Chem. Soc., 80, 4395-4404 (1958).

Uyeo, S., Fales, H. M., Highet, R. J. and Wildman, W. C., Oxohaemanthidine: A Bicyclic Lactam Possessing a Bridgehead Nitrogen, J. Am. Chem. Soc., 80, 2590 (1958).

Serial No. NHI-165

1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Alkaloids. Isolation and Structure Determination.

Principal Investigator: S. M. Goodwin, Ph.D.

Other Investigators: A. A. Velasquez (Technical in part)

Cooperating Units: None.

Man Years:
Total: .75
Professional: .50
Other: .25

Patient Days:
None

Project Description:

Progress during the Past Year

(1) In continuing the studies of the alkaloids of Lunasia amara, three minor alkaloids were isolated for the first time; they are hydroxylunacridine, hydroxylunidine, and hydroxylunine. Structures were assigned to them on the basis of analytical and spectral evidence, including NMR. The structure of hydroxylunacridine was elucidated unequivocally by standard chemical degradative reactions; this is the first optically active Lunasia alkaloid to have a complete chemical structure proof.

(2) The validity of the proposed structure of lunamarine (see 1957 report) was tested by the synthesis of the compound having this structure; the synthetic compound was identical in all respects with the natural product. An attempt to synthesize the desmethoxy analog of lunacridine by a six-step synthesis was investigated.

Direction of Current Research:

All of the research under Dr. E. C. Horning has been discontinued. Future work will be under Dr. W. C. Wildman.

Part B included. Yes

Serial No. NHI-165

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B: Publications

In press:

Goodwin, S. M., Smith, A. F. and Horning, E. C. Alkaloids of *Ochrosia elliptica*, Labill. J. Am. Chem. Soc., 81, 000 (1959).

Goodwin, S. M. and Horning, E. C. Alkaloids of *Lunasia amara* Blanco. The Structure of Lunacrine. J. Am. Chem. Soc., 81, 000 (1959).

Serial No. NHI-166

1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Nuclear Magnetic Resonance Studies on Alkaloids.

Principal Investigator: S. M. Goodwin, Ph. D.

Other Investigator: None.

Cooperating Units: Dr. J. N. Shoolery and Mr. L. F. Johnson, Varian Associates, Palo Alto, California

Man Years
Total: .50
Professional: .50
Other:

Patient Days
None.

Project Description:

Progress during Past Year

An evaluation has been made concerning the applicability of nuclear magnetic resonance spectroscopy to the elucidation of the structures of alkaloids. The compounds selected for study were almost exclusively those alkaloids which had been isolated and studied chemically by the investigator. The NMR studies were an enormous success and indicated that nuclear magnetic resonance spectroscopy is an important new tool to be added to other physical methods used in obtaining evidence pertaining to the structures of organic compounds. Some of the results are summarized as follows:

(1) The question of whether ellipticine and methoxyellipticine contain one ethyl or two methyl groups (chemical analysis gave negative results) was solved by NMR; the answer being two methyl groups.

(2) The alkaloids A and C of Ocotea leucoxylon had been shown to be aporphine alkaloids having six and five oxygen substituents, respectively. These two alkaloids and three others of the aporphine type having known structures were studied. New insight of important significance into the nature of aporphine alkaloids, in general, was obtained. The twenty-five possible structures of alkaloid A may be narrowed down

to very strong evidence for one with a second possibility not completely ruled out and similarly on the basis of NMR evidence alone, the fifty-five theoretically possible structures of alkaloid C have been narrowed down to two. Other considerations may be invoked to make the final choice; although if the NMR studies were extended to a few other known aporphine alkaloids in order to get more data for frequency assignments, the final choice could be made on the basis of NMR evidence alone. The spectrum of an unknown aporphine alkaloid sent to me by chemists at the Squibb Company of Argentina, provided evidence that the structure proposed by the Argentinians was incorrect; two possibilities were suggested, one of which can be selected as agreeing with earlier chemical evidence.

(3) The fourteen alkaloids isolated here from Lunasia amara have been examined. The outstanding results are the interpretation of the spectrum of lunacrine which enables its complete structure to be written, the application of this knowledge to the minor alkaloid lunine which gives the total structure determination of this alkaloid from merely the analytical data and the NMR spectrum, the structure proofs of hydroxy-lunacridine and hydroxylunidine were accomplished by NMR, and finally, the positions of the hydroxyl groups in hydroxylunacrine and hydroxylunine may be assigned on the basis of their NMR spectra.

(4) Several structures have been proposed by various investigators for the alkaloid conessine. Only one of these was in agreement with the NMR spectrum. (Subsequently, the partial synthesis of conessine was achieved by Dr. E. J. Cory which proved its structure; it was the one indicated by NMR).

Direction of Current Research

Attention is being given to preparing the NMR studies for publication; additional data must be obtained on many of the alkaloids. Other aporphine alkaloids are being collected with the assistance of Dr. Inubushi and Dr. E. Schlittler of Ciba. It is hoped that it will be possible to use this technique in studying unsolved problems in the structures of other alkaloids, such as questions in stereochemistry of the quinine alkaloids and in the β-yohimbine series.

Part B included: Yes.

Serial No. NHI-166

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B. Publications.

Goodwin, S., Shoolery, J. N. and Johnson, L. F. Significance of the Nonequivalence of Methylenedioxy Hydrogen Hydrogen Nuclei in the Nuclear Magnetic Resonance Spectra of Aporphine Alkaloids. Proc. Chem. Soc., 406 (1958).

Shoolery, J. N., Goodwin, S. and Johnson, L. F. Nuclear Magnetic Resonance Spectra of Alkaloids. I. The Complete Structures of Lunacrine and Lunine. J. Am. Chem. Soc., 81, 000 (1959).

Serial No. NHI-167

1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies on Alkaloids. To discover new Alkaloids of potential therapeutic value and to elucidate their structures.

Principal Investigator: Perola Zaltzman (Visiting Scientist)

Other Investigators: None

Cooperating Units: Dr. Bernice G. Schubert, Plant Industry Station, U. S. Department of Agriculture, Beltsville, Md. Plant Identifications.

Man Years:
Total: .40
Professional: .40
Other:

Patient Days:
None

Project Description:

Progress during the Past Year:

A. Himatandra belgraveana (Himatandraceae)

This plant, among others, was sent to us from New Guinea as part of a program of investigation regarding the "Kuru" disease that has been killing hundreds of natives of that island.

The illness was described by Drs. Gajdusek and Zigas in a report for the New England Journal of Medicine (Nov. 1957), in which they stated that the extensive neurologic degeneration found on histologic study strongly suggested some toxic factor.

A crude extract of the alkaloids of the bark of Himatandra showed a very strong physiological activity in rats (convulsions ending in death). Fractional crystallization and chromatography on alumina produced two main alkaloids, one of which was himbacine: Aust. J. Chem. 9, 283 (1956), $C_{22}H_{35}O_2N$, m.p. 136° $[\alpha]_{589}^{25}$ + 62.5° [0.510% in $CHCl_3$].

Himbacine retained the activity observed in the crude extract while the other alkaloid, m.p. 217-223°, not identified, was inactive.

The lethal dose of himbacine hydrochloride was 150 mg./kg. when the drug was injected intraperioneally in mice.

B. Annona sp. (Annonaceae)

This project had been interrupted, pending the arrival of a new shipment of plant material.

The plant had shown a marked hypotensive action in dogs and two alkaloids not previously reported had been isolated and characterized (report of 1957).

However, the new samples of A. cherimolia and A. reticulata showed no evidence of the alkaloids named X and Y. This could be attributed to the fact that they were collected in a different area.

Chromatography on silicic acid column did not achieve any separation. One of the major fractions was subjected to counter-current distribution and the fractions examined by U.V. absorption and paper chromatography. Some purification was achieved but no crystalline material was obtained..

Direction of Current Research:

A. Himatandra belgraveana. This work has been terminated.

B. Annona sp. Due to the small yield of alkaloidal material and to the difficulty in obtaining more plant material, the project was discontinued.

Part B included. Yes No [x]

Serial No. NHI-168

1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

FHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Synthesis, Degradation and Interconversions of the Amaryllidaceae Alkaloids.

Principal Investigator: Henry M. Fales, Ph.D.

Other Investigators: None.

Cooperating Units: None.

Man Years:
Total: 1
Professional: 1
Other:

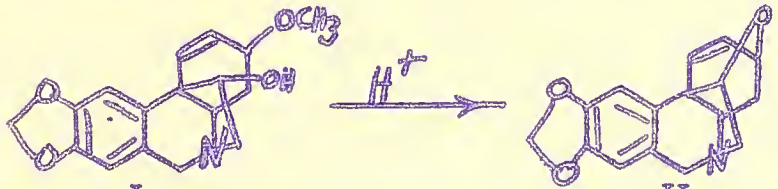
Patient Days:
None

Project Description:

Progress During the Past Year:

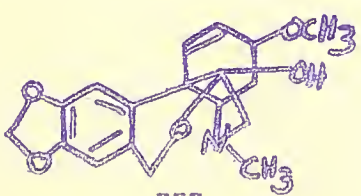
Progress has been made in the interconversions of many of the Amaryllidaceae alkaloids and certain steric relationships have evolved.

The structure of haemanthamine (natalensine) has been found to be I.

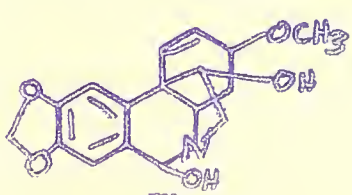


I II

It was converted to aponatalensine (II) by dilute acid. The dihydro derivative of aponatalensine (II) has been prepared from haemanthidine (IV) which in turn has been converted to tazettine (III). Therefore, the nucleus of the 3 alkaloids is identical, and necessarily a trans B:C ring fusion exists. Further the methoxyl and phenyl groups



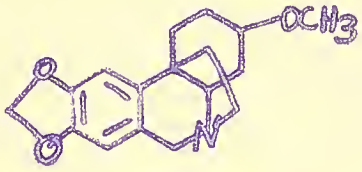
III



IV

bear the same relationship in all 3 alkaloids.

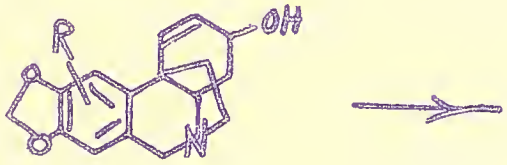
The dihydro derivative of haemanthamine has been converted by thionyl chloride and lithium aluminum hydride to the optical antipode of dihydro buphanisine (V), which is a member of another group of alkaloids



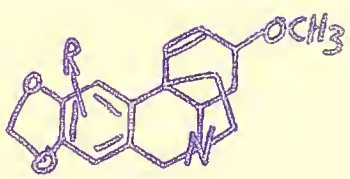
V

which includes buphanidrine, powelline, buphanamine, crinine, crinamidine and undulatine. Therefore the steorochemistry of these two large classes of Amaryllidaceae alkaloids is known and one class is related enantiomerically to the other. Since the buphanisine class uniformly possess analgesic properties while the other does not, an assymetric mode of analgesic action is indicated.

Relationships have been found to exist among the other alkaloids of the buphanisine class. Powelline (VI) has been converted to buphanidrine (VII), crinine (VIII) to buphanisine (IX), and crinamidine (X) to undulatine

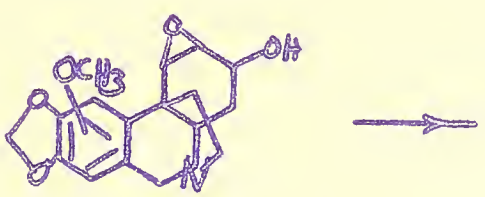


VI R = OCH_3
VIII R = H

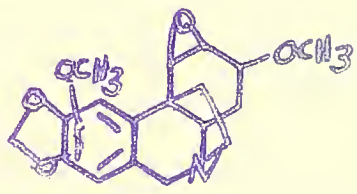


VII R = OCH_3
IX R = H

(XI) by the use of potassium metal and methyl p-toluene sulfonate, a com-



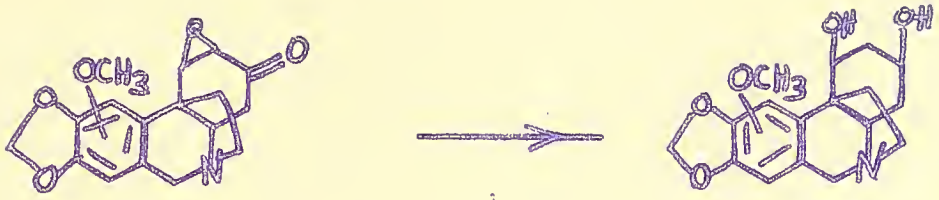
X



XI

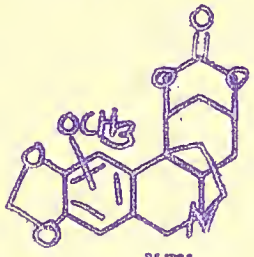
bination designed to methylate alcohols without quaternizing tertiary amines. This reagent also preserves the configuration of the hydroxyl group involved. Powelline (VI) has been converted via its epoxyoxo-

derivative (XII) to haemanthine (XIII), the key alkaloid on which the



XII XIII

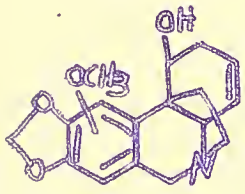
configuration of all the groups in ring C is based. The hydroxyl groups in ring C are cis diaxial since a cyclic carbonate (XIV) was formed on



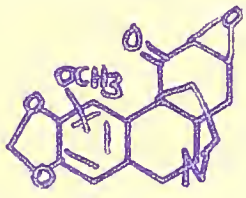
XIV

treatment of XIII with phosgene.

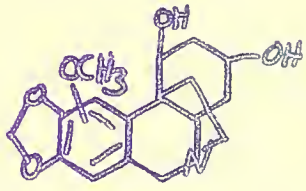
Finally buphanamine has been found to possess structure XV since it was converted to the epoxyoxo derivative (XVI) on treatment with



XV



XVI



XVII

chromic acid-pyridine followed by alkaline hydrogen peroxide. This compound afforded dihydrocrinamidine (XVII) with lithium aluminum hydride. This same compound was obtained also by the action of lithium aluminum hydride on crinamidine. The double bond has been placed in the homoallylic position because of failure of the hydroxyl group to oxidize with manganese dioxide.

Direction of Current Research:

Final minor points concerning the stereochemistry and abnormal reactions of several of the above alkaloids will be investigated further. The structures of montanine and coccinine are being clarified and their relationship to haemanthamine is under investigation. Larger quantities of the optical antipode of buphanisine and other derivatives are being pre-

pared for testing for analgesic properties by Dr. N. Eddy of NIAMD. Synthetic routes are being sought for the alkaloids. The alkaloid tecomanine is under further investigation.

Part B included. Yes

Serial No. NHI-168

1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B: Publications

Fales, H. M. and Wildman, W. C., Interconversions of Amaryllidaceae Alkaloids by Sodium and Amyl Alcohol, J. Am. Chem. Soc. 80, 4395-4404 (1958).

Fales, H. M. and Wildman, W. C., Structure of Haemanthamine, Chemistry and Industry, 561-562 (1958).

Uyeo, S., Fales, H. M., Highet, R. J. and Wildman, W. C., Oxohaemanthidine: A Bicyclic Lactam Possessing a Bridgehead Nitrogen, J. Am. Chem. Soc. 80, 2590-2591 (1958).

Fales, H. M. and Wildman, W. C., Stereochemistry of the 5,10b-Ethanophenanthridine Alkaloids of the Amaryllidaceae, J. Am. Chem. Soc. (In press), (Dec. 1958).

Serial No. NHI-169

1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Isolation of Alkaloids, Glycosides and Other Constituents from Plant Materials.

Principal Investigator: David L. Rogerson, Jr.

Other Investigators: James D. Link (Technical)
Douglas L. Johnson (Technical)

Cooperating Units: Dr. John C. Keresztesy, NIAMD, Henry E. Lutterlough and James M. Miles. Large Scale equipment.
Dr. Bernice G. Schubert, Plant Industry Station, U. S. Department of Agriculture, Beltsville, Md. Plant Identifications and Procurements.

Man Years:
Total: 1.95
Professional: .50
Other: 1.45

Patient Days:
None

Project Description:

Progress During the Past Year:

Plant materials weighing in excess of 400 lbs. have been processed for alkaloids. A total of 39 samples were processed of which 29 were new observations and 10 repeats of previously extracted varieties. Included in the processing of new materials were 16 plants resulting from the Mexican program.

In addition to the 234 plant materials that have been processed for alkaloids to date, 16 materials have been partially or completely processed for glycosides. Also, a total of 284 supplementary shipments of previously procured materials has been received. This is an increase of 13 since January 1, 1958.

Processing for desired constituents other than alkaloids and glycosides has been conducted on 9 plant samples.

Direction of Current Research:

Efforts are being made to obtain Mexican plant materials in bulk

which have given favorable initial observations. Methods will be developed for the isolation of the active components from materials which indicate strong physiological activity as observed in the glycoside testing program.

Part B included. No

Serial No. NHI-170

1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Isolation of Constituents from Human Urine and Animal Tissue.

Principal Investigator: David L. Rogerson, Jr.

Other Investigator: James D. Link (technical)
Douglas L. Johnson (technical)

Cooperating Units: None.

Man Years
Total: .30
Professional: .10
Other: .20

Patient Days
None.

Project Description:

Progress during the Past Year

Organic constituents, mainly acids and bases, have been isolated from human urine totaling 500 l., which is an increase of 300 l. since last reported. The urine samples were obtained locally from male donors.

In addition to the previously reported 1200 pounds of fresh, frozen veal brains and 20 pounds of frozen pork chitterlings processed for this section, 65 pounds of beef hearts have been processed for the National Heart Institute's Laboratory of Chemical Pharmacology.

Direction of Current Research

Large scale processing of fresh, frozen veal brains will be resumed upon completion of current researches for improved isolation techniques. Also, the isolation of substances from human urine recently collected from employees will be continued.

Page B included: No.

Serial No. NHI-171

1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Testing of Plant Materials for Alkaloids and Glycosides.

Principal Investigators: David L. Rogerson, Jr.

Other Investigators: James D. Link (Technical)
Douglas L. Johnson (Technical)

Cooperating Units: B. G. Schubert, Plant Industry Station, U. S. Department of Agriculture, Beltsville, Md. Plant Identifications and Procurements.

Man Years:
Total: .45
Professional: .30
Other: .15

Patient Days:
None

Project Description:

Progress During the Past Year:

To date, 3355 plant samples, which includes 524 Herbarium specimens, have been tested for alkaloids and 841 samples (25%) gave positive results. The total number of plants screened represents an increase of 260 analyses since December 31, 1957, 228 of which were made by this investigator in Mexico.

In addition to the alkaloid determinations, 54 of the above samples have been screened for glycosides and the results on 5 more samples are pending. Physiological activity was observed for 35 of the plants tested through subcutaneous injection of mice.

Direction of Current Researches:

Attention will be focused on a more standardized procedure for the determination of physiologically active, water soluble, non-alkaloidal components using existing plant supplies.

Part B included. No

Serial No. NHI-172

1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A:

Project Title: Preparations and Special Isolations.

Principal Investigator: David L. Rogerson, Jr.

Other Investigator: James D. Link (technical)
Douglas L. Johnson (technical)

Cooperating Units: Dr. John C. Keresztesy and Staff, (NIAMD Serial No.). Large scale equipment.

Man Years
Total: .15
Professional: .05
Other: .10

Patient Days
None.

Project Description:

Progress during the Past Year

Silicic acid and deactivated charcoal (8% stearic acid) in pound quantities have been prepared for chromatographic uses and separations and purifications of relatively large, crude, alkaloidal extracts have been conducted by chromatographic means.

The 100 gallon fermentation unit has been employed to grow bacteria on thirteen occasions for eight individuals representing three Institutes; Heart, Arthritis and Mental Health.

Special isolations include the extraction of 1800 ml. red blood cells and the extraction of a blood factor from two quantities of whole blood.

Direction of Current Research

Organic compounds and substances will be synthesized and prepared in all desired quantities as directed by the needs of the Section. Also, a standardized operating procedure for the Fermentation Unit will be developed along with minor changes and additions.

New procedures and techniques will be developed and employed to cope with the increase in requests for special, large-scale isolations work.

Part B included: No.

Serial No. NHI-173
1. Laboratory of Chemistry of Natural Products
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A:

Project Title: The Isolation of Andromedotoxin from Rhododendron and Related Genera.

Principal Investigator: David L. Rogerson, Jr.

Other Investigator: James D. Link (technical)
Douglas L. Johnson (technical)

Cooperating Units: Dr. John C. Keresztesy and Henry E. Lutterlough, NIAMD (Serial No.). Large scale isolation equipment and its operation.

Man Years
Total: .15
Professional: .05
Other: .10

Patient Days
None.

Project Description:

Progress during Past Year

The isolation of Andromedotoxin has been discontinued and the materials received to date for processing in bulk are as follows: 7938 lbs. Rhododendron maximum (an increase of 516 lbs.), 705 lbs. Kalmia latifolia and 350 lbs. Kalmia angustifolia, var. caroliniana. In addition, 23 members of the Ericaceae family have been investigated for Andromedotoxin content on a one pound level or less.

Direction of Current Research

None.

Part B included: No.

Form No. ORP-2
Oct. 1957

PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
Laboratory of Chemical Pharmacology

Estimated Obligations for FY 1959

Total:	$517,944
Direct:	$393,000
Reimbursements:	$124,944

Serial No. NHI-174
1. Chemical Pharmacology
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Serum Transaminase and Alkaline Phosphatase Levels after Large Doses of Norepinephrine and Epinephrine in Dogs

Principal Investigator: Dr. Harriet M. Maling

Other Investigators: Mrs. Martha A. Williams
Mr. Duffy E. McBrayer

Cooperating Unit: Dr. Benjamin Highman, National Institute of Arthritis and Metabolic Diseases

Man Years (calendar year 1958):
Total: 0.40
Professional: 0.15
Other: 0.25

Patient Days (calendar year 1958):
None

Project Description:

Objectives: To determine whether large doses of norepinephrine and epinephrine in dogs cause elevations in serum glutamic oxalacetic transaminase (SGO-T) and serum glutamic pyruvic transaminase (SGP-T). Simultaneous measurements of SGO-T and SGP-T have been used in the differential diagnosis of myocardial and hepatic damage. Cardiac damage, evidenced by ventricular hypersensitivity and widespread myocardial fatty changes with foci of hemorrhage and necrosis, was found in this laboratory for several days following an intravenous infusion of a large dose of norepinephrine or epinephrine in unanesthetized dogs (Maling and Highman, Am. J. Physiol. 194:590, 1958). Studies from other laboratories indicate the occurrence of both myocardial and hepatic damage following large doses of catechol amines. In this project, serum alkaline phosphatase has also been measured as another indicator of possible hepatic damage.

Methods Employed: Unanesthetized healthy mongrel dogs were given a large dose of norepinephrine or epinephrine either by continuous intravenous infusion or by subcutaneous injection of 1 mg/kg epinephrine-in-oil. SGO-T and SGP-T were measured by the colorimetric method described by Reitman and Frankel (Am. J. Clin. Path. 28:56, 1957). Serum alkaline phosphatase was measured by the procedure of Bogdanski,

Serial No. NHI-174

using the colorimetric method of Fiske and Subbarow to determinethe phosphate liberated. Most of the dogs used for pathologic study were killed with sodium pentobarbital 1-2 days after a large dose of norepinephrine or epinephrine. Specimens from nearly all organs were fixed in 10 per cent formalin buffered to pH 7.0. Routine paraffin sections were stained with hematoxylin and eosin. Frozen sections were stained for neutral fat with Oil red O. The microscopic findings were compared with those seen in dogs given saline infusions.

Major Findings: An intravenous infusion in conscious dogs of a large dose of norepinephrine (0.51-0.85 mg/kg) or epinephrine (0.55-0.92 mg/kg is followed by an elevation in SGO-T which reaches a peak in about 6 hours and subsides within 2-3 days. SGP-T and serum alkaline phosphatase increase more gradually and subside more slowly. Pathologic studies confirm the myocardial and hepatic damage suggested by the elevated serum enzyme levels. Subcutaneous injection of 1 mg/kg epinephrine-in-oil produces less constant and severe pathologic changes, but is followed by a gradual increase during the first day in SGO-T, SGP-T and serum alkaline phosphatase with peak levels considerably higher than those following the intravenous infusions. The adrenergic blocking agent Dibenzyline prevents the rise in serum transaminases but not the rise in serum alkaline phosphatase if injected intravenously in a dose of 2 mg/kg one hour before administration of epinephrine-in-oil.

Significance to the Program of the Institute: These findings in dogs suggest the possibility of similar findings in man. Norepinephrine is widely used to maintain arterial pressure during coronary shock and SGO-T is used diagnostically in myocardial infarction. If norepinephrine causes an elevation of SGO-T in man, the interpretation of elevated SGO-T levels after coronary occlusion may be confusing.

These findings suggest that large doses of norepinephrine and epinephrine-in-oil should be used cautiously, because of the possibility of causing cardiac and hepatic damage.

Proposed Course of Project: This project has been completed.

Part B included: Yes

Serial No. NHI-174

Publications:

Highman, B., H.M. Maling and E.C. Thompson. Serum transaminase and alkaline phosphatase levels after large doses of norepinephrine and epinephrine in dogs. Am. J. Physiol., in press.

Honors and Awards: None

Serial No. NHI-175
1. Chemical Pharmacology
2. Cell Permeability
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Entrance of Substances into the Central Nervous System

Principal Investigator: Dr. Lewis S. Schanker

Other Investigator: Dr. Hermann Kurz

Cooperating Units: Dr. Kurz' salary was paid by a fellowship from the Max Kade Foundation

Man Years (Calendar Year 1958): Patient Days: None
Total: 1.00
Professional: 1.00
Other: 0

Project Description:

Objectives: To determine what factors govern the rates at which drugs pass from the bloodstream into the central nervous system.

Methods Employed: Dogs were anesthetized with chloralose and urethan, a polyethylene catheter was placed in the cisterna magna for the collection of cerebrospinal fluid (CSF), and various drugs were administered via the femoral vein. Concentrations of drug in the CSF and in plasma water were measured in samples collected at various times. The relative rates at which various drugs enter the CSF were compared graphically by plotting the CSF to plasma water concentration ratios against time.

Major Findings: The time required for various drugs to reach an equilibrium CSF:plasma concentration ratio of about 1.0 ranged from several minutes to more than 5 hours. Some drugs entered the CSF so slowly that they were barely detectable even 5 hours after beginning the intravenous administration of the drug.

Serial No. NHI-175

The degree of ionization and the lipid-solubility of the unionized drug molecule appear to be the most important properties of a drug which determine its rate of passage across the blood-CSF barrier. In general, weak organiz electrolytes entered the CSF much more rapidly than the stronger, highly ionized organic electrolytes. Among the unionized drugs studied, those having the highest lipid-solubility penetrated most rapidly and those which were poorly soluble in lipids penetrated at much slower rates.

The results suggest that the blood-CSF barrier is lipoid in character; it is preferentially permeable to the unionized, lipid-soluble form of foreign organic compounds.

Significance to the Program of the Institute: An understanding of the factors governing the passage of drugs into the central nervous system should lead to the selection of therapeutic agents, as well as to the synthesis of new drugs, which will most readily reach their site of action in suitable concentration to exert the desired pharmacologic effect.

Proposed Course of Project: (1) Investigations concerning the locus of the blood-brain barrier. (2) Investigations dealing with the distribution of drugs in the central nervous system. (3) Investigations dealing with the formation and fate of cerebrospinal fluid. (4) Study of the barrier between cerebrospinal fluid and nervous tissue of the central nervous system.

Part B included: No

Serial No. NHI-176
1. Chemical Pharmacology
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Studies concerning the role of epinephrine and norepinephrine in lipid mobilization and deposition.

Principal Investigator: Dr. Harriet M. Maling

Other Investigators: Mr. William M. Butler, Jr.
Mrs. Martha A. Williams

Cooperating Units: Dr. Benjamin Highman, National Institute of Arthritis and Metabolic Diseases

Man Years (Calendar Year 1958): Patient Days: None
Total: 1.25
Professional: 0.15
Other: 1.1

Project Description:

Objectives: To elucidate the role of epinephrine and norepinephrine in the mobilization and deposition of fat. The demonstration in this laboratory of myocardial fatty changes in dogs after intravenous infusions of large doses of epinephrine or norepinephrine and the prevention of marked fatty changes by the prior administration of the adrenergic blocking agent Dibenzyline both suggest that epinephrine may be involved in the deposition of fat. Other investigators have shown that adrenalectomized animals do not develop fatty livers under circumstances producing fatty infiltration in intact animals (Ramsey and Goldstein, Physiol. Rev. 37: 55, 1957). If epinephrine is essential to fat deposition in the liver and other organs, adrenergic blocking agents would be anticipated to be effective in preventing fatty infiltration of the liver by diverse agents. Wool and co-workers (Am. J. Physiol. 178: 427, 1954) have shown that ergotamine partially prevents the fatty livers which can be induced by ethionine in fasting female rats. We are studying the effectiveness of various adrenergic blocking agents in inhibiting fatty infiltration of the liver. If adrenergic blocking agents, with different chemical structures, all are effective in inhibiting fatty infiltration of the liver, it is probable that epinephrine plays an essential role in fat mobilization and/or deposition.

Serial No. NHI-176

Methods Employed: The triglyceride content of tissue is measured by a modification of the direct method of van Handel and Zilversmit (J. Lab. & Clin. Med. 50: 152, 1957). Total lipids are determined gravimetrically. Phospholipid concentrations are obtained by multiplying lipid phosphorus values by 25. Total cholesterol is measured by the method of Abel and co-workers (J.B.C. 195:357, 1952).

In some of the animals, tissues obtained at autopsy are fixed in 10 per cent formalin buffered to pH 7.0. Routine paraffin sections are stained with hematoxylin and eosin. Frozen sections are stained for neutral fat with Oil red O.

Major Findings: In dogs killed the day after intravenous infusion of large doses of epinephrine or norepinephrine, frozen sections of the myocardium stained with Oil red O reveal fatty changes. The triglyceride content of normal dog heart varies considerably from dog to dog and we are still not sure whether infusions of epinephrine or norepinephrine increase the triglyceride content of dog heart significantly. The results of many determinations will have to be evaluated statistically.

Preliminary data indicate that the adrenergic blocking agents Dibenzyline, Diberamine and ergotamine are all effective in preventing the fatty livers produced in fasting rats by the subcutaneous injection of carbon tetrachloride.

Significance to the Program of the Institute: This project should increase our understanding of the processes involved in lipid mobilization and deposition. Indirectly, the project may give clues to the nature of hepatic disease and atherosclerosis.

Proposed Course of Project: A study will be made of the effectiveness of adrenergic blocking agents in preventing fatty infiltration of the liver and other tissues after various procedures which are known to produce fatty livers. These procedures include the administration of large doses of ethanol (Luzio, Am. J. Physiol. 194: 453, 1958), ethionine in fasting female rats (Wool and Goldstein, Am. J. Physiol. 175: 303, 1953), and phosphorus.

In addition to the three adrenergic blocking agents already tested, this study will be extended to include regitine, priscoline, and dichlorophenyl-2-isopropylaminoethanol (Powell and Slater, J. Pharmacol. & Exp. Therap. 122: 480-488, 1958). Studies will also be made on adrenalectomized rats whose adrenal glands have been depleted of catechol amines by prior treatment with reserpine.

Part B included: yes

Serial No. NHI-176

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Maling, H. M. and B. Highman. Exaggerated ventricular arrhythmias and myocardial fatty changes after large doses of norepinephrine and epinephrine in unanesthetized dogs. Am. J. Physiol. 194: 590-596, 1958.

Honors and Awards: None

Serial No. NHI-177
1. Chemical Pharmacology
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: The Effect of Coronary Artery Occlusion in Reserpinized Dogs

Principal Investigator: Dr. Harriet M. Maling

Other Investigators: Mr. Victor H. Cohn, Jr.
Mrs. Alice Williams
Mr. Duffy E. McBrayer

Cooperating Units: Dr. Benjamin Highman, National Institute of Arthritis and Metabolic Diseases

Man Years (calendar year 1958): Patient Days: None
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Objectives: To determine whether the presence of norepinephrine in the myocardium is necessary for the development of spontaneous ectopic activity and prolonged cardiac hypersensitivity following coronary artery occlusion in dogs. To determine whether the absence of norepinephrine in the myocardium after reserpine will modify significantly the histologic appearance of the infarct.

Methods Employed: Reserpine (0.25 mg/kg) was injected intravenously in normal dogs. The anterior descending coronary artery was ligated by the two stage occlusion procedure of Harris (Circulation 1:1318, 1950) 20-30 hours after administration of reserpine. The dogs were tested for the presence of spontaneous ectopic activity and for cardiac hypersensitivity for a number of days after occlusion, as in a previous study (Maling and Moran, Circulation Research, 5: 409, 1957). At varying times after the administration of reserpine, the dogs were killed for pathologic examination.

Serial No. NHI-177

Non-infarcted muscle and myocardium from other reserpinized dogs not subjected to coronary occlusion were analyzed for norepinephrine content. Myocardial infarcts were examined grossly, and fixed in 10 per cent formalin buffered to pH 7.0. Frozen sections of the myocardium were stained with neutral fat with Oil red O.

Major Findings: The mortality after coronary artery occlusion is approximately the same in reserpinized dogs as in normal dogs. Two of 14 reserpinized dogs died from ventricular fibrillation within minutes after coronary artery occlusion. This may be compared with 10 deaths within 30 minutes after occlusion in 101 operations on normal dogs.

Although in the reserpinized dogs, the heart was depleted of norepinephrine, spontaneous ectopic activity developed as usual the day after coronary artery occlusion. The duration of the spontaneous ectopic activity was within the usual limits. Cardiac hypersensitivity could be demonstrated after the spontaneous ectopic activity had disappeared. These findings indicate that the spontaneous ectopic activity and the prolonged state of cardiac hypersensitivity cannot be explained in terms of the release of norepinephrine from the infarcted muscle during necrosis and its absorption on the neighboring normal muscle.

The gross and microscopic appearance of the infarcts was not significantly different in the reserpinized dogs from infarcts produced in non-reserpinized dogs. Fatty changes were present surrounding the infarcted muscle in reserpinized dogs, just as in the control dogs.

Significance to the Program of the Institute: Our findings indicate that the catechol amines in the myocardium do not contribute significantly to the spontaneous ectopic activity and the cardiac hypersensitivity following coronary artery occlusion.

Proposed Course of Project: These findings are being prepared for publication.

Part B included: Yes

Serial No. NHI-177

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Shore, P. A., V. H. Cohn, Jr., B. Highman and H. M. Maling: Distribution of norepinephrine in the heart. Nature 181:848, 1958.

Honors and Awards: None

Serial No. NHI-178
1. Chemical Pharmacology
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: High Altitude Tolerance of Normal Dogs and Dogs with Myocardial Infarcts

Principal Investigator: Dr. Harriet M. Maling

Other Investigator: Mrs. Martha A. Williams

Cooperating Units: Dr. Benjamin Highman, National Institute of Arthritis and Metabolic Diseases

Man Years (calendar year 1958): Patient Days: None
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Objectives: To determine whether myocardial infarction impairs appreciably the ability of dogs to withstand a simulated exposure to a high altitude.

Methods Employed: Normal dogs and dogs with myocardial infarcts produced by two-stage occlusion of the anterior descending coronary artery were placed in halters and tied with leashes to the walls of a large decompression chamber. Lead II electrocardiograms of some of the dogs were recorded outside the chamber on Sanborn Viso-Cardiettes, with all connections made through a box on the wall of the chamber. Electrodes were held in place on the dogs' legs with elastic bandages. The connecting leads were brought out through the halter in such a way that the dogs were able to stand, sit down, and walk unless restricted by the leash. The physical activity and respiration of each dog were observed by frequent intervals through windows in the walls of the decompression chamber.

Simulated altitudes of 34,000 and 38,000 feet were obtained by decompression within the chamber in a stepwise manner.

Serial No. NHI-178

Electrocardiographic responses to intravenous test doses of norepinephrine were recorded on some dogs one or more days before and the day after exposure to a simulated high altitude.

Autopsies were performed shortly after recompression of the chamber on dogs which died during exposure. Most of the survivors were killed with sodium pentobarbital 1 - 2 days after exposure. Tissues were fixed in 10 per cent formalin buffered to pH 7.0. Paraffin sections were stained with hematoxylin and eosin. Frozen sections were stained for the presence of neutral fat with Oil red O.

Major Findings: Most unanesthetized normal dogs survive single exposures for 3 - 3.5 hours to simulated altitudes of 34,000 and 38,000 feet. Three or more days after coronary occlusion, dogs with large myocardial infarcts showed only a slight reduction in tolerance to simulated high altitudes. Respiratory and heart rates increased markedly in all dogs at high altitudes. Throughout the exposures, ectopic ventricular beats were recorded frequently in most of the dogs with infarcts and rarely in normal dogs. Deaths during exposure among normal and sham-operated dogs were from respiratory failure. Two deaths from ventricular fibrillation were recorded in dogs with infarcts. Exposure to high altitudes did not modify significantly either the gross or histologic appearance of the infarcts.

Significance to the Program of the Institute: Our findings in dogs suggest that, after coronary infarction, persons may show little or no reduction in tolerance to high altitudes. A reappraisal of flight restrictions on such individuals is desirable.

Proposed Course of Project: This project has been completed.

Part B included: Yes

Serial No. NHI-178

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Maling, H. M., and Benjamin Highman. High altitude tolerance of normal dogs and dogs with myocardial infarcts. Am. J. Physiol., in press.

Honors and Awards relating to this project: None

Serial No. NHI-179
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Studies on the Identification and Distribution of Catecholamines in the Body

Principal Investigators: Dr. P. A. Shore
Dr. D. G. Bogdanski

Other Investigators: Dr. V. H. Cohn
Mr. R. Kuntzman

Cooperating Units: Dr. H. Highman, NIAMD

Man Years (Calendar year 1958): Patient Days: None
Total: 1-5/8
Professional: 5/8
Other: 1

Project Description:

Objectives: Because of the great interest in possible functions of catecholamines in the brain, heart and other organs, a study of the distribution of catecholamines in the body and within various organs has been carried out.

Major Findings: The simple and sensitive chemical method, described in the 1957 report, for the estimation of norepinephrine and epinephrine in tissues, has been adapted to permit the estimation of small fractions of a microgram.

In collaboration with Dr. Harriet Maling of this laboratory and Dr. B. Highman of NIAMD, a study has been carried out of the distribution of norepinephrine in dog heart. It was found that while little epinephrine occurred in heart, large concentrations of norepinephrine, about 2 μg/g, are present. Especially high concentrations, about 2.7 μg/g, occurred in the atria while the concentration in the ventricles was only about one-half this figure. There was some suggestion that the endocardium may be devoid of norepinephrine.

Serial No. NHI-179

Studies on the distribution of norepinephrine within the brain show that it is essentially confined within certain broad areas of grey matter and is not present in tracts of nerve fibers or confined to discrete nuclei. There appeared to be an association of norepinephrine with the reticular formation. This is of interest in view of the hypothesis that a catecholamine may be a chemical mediator of this system.

Significance to the Program of the Institute: These studies are of significance in that they are an aid to a better understanding of some of the basic mechanisms underlying the nervous control of the cardiovascular system.

Proposed Course of Project: Further studies of the distribution of catecholamines in the brain are planned in an effort to associate these substances with a definite function in the brain.

Part B included: Yes

Serial No. NHI-179

Publications:

Shore, P.A., Cohn, V.H., Highman, B., and Maling, H.M., Distribution of Norepinephrine in the Heart. Nature 181, 848, 1958.

Shore, P.A., A simple technique involving solvent extraction of the estimation of norepinephrine and epinephrine in tissues. Pharmacol. Rev., in press.

Shore, P.A., and Olin, J.S., Identification and chemical assay of norepinephrine in brain and other tissues. J. Pharmacol., 122, 295, 1958.

Honors and Awards: None

Serial No. NHI-180
1. Chemical Pharmacology
2. Drug Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: The Enzymatic Oxidation of Nicotine

Principal Investigator: Dr. Howard B. Hucker

Other Investigator: None

Cooperating Unit: None

Man Years (calendar year 1958):
Total: 1
Professional: 1
Other: 0

Patient Days (calendar year 1958):
None

Project Description:

Objectives: To study the enzymatic system(s) by which nicotine is metabolized in animal organisms.

Methods Employed: A differential solvent extraction method for the simultaneous determination of microgram quantities of nicotine and cotinine.

Major Findings: Cotinine was shown to be a major product of nicotine oxidation by a rabbit liver preparation consisting of microsomes and soluble fraction. Approximately 50 per cent of the added nicotine is converted to cotinine in this system. We have previously noted that TPNH and oxygen are required for the reaction, which is catalyzed by microsomes.

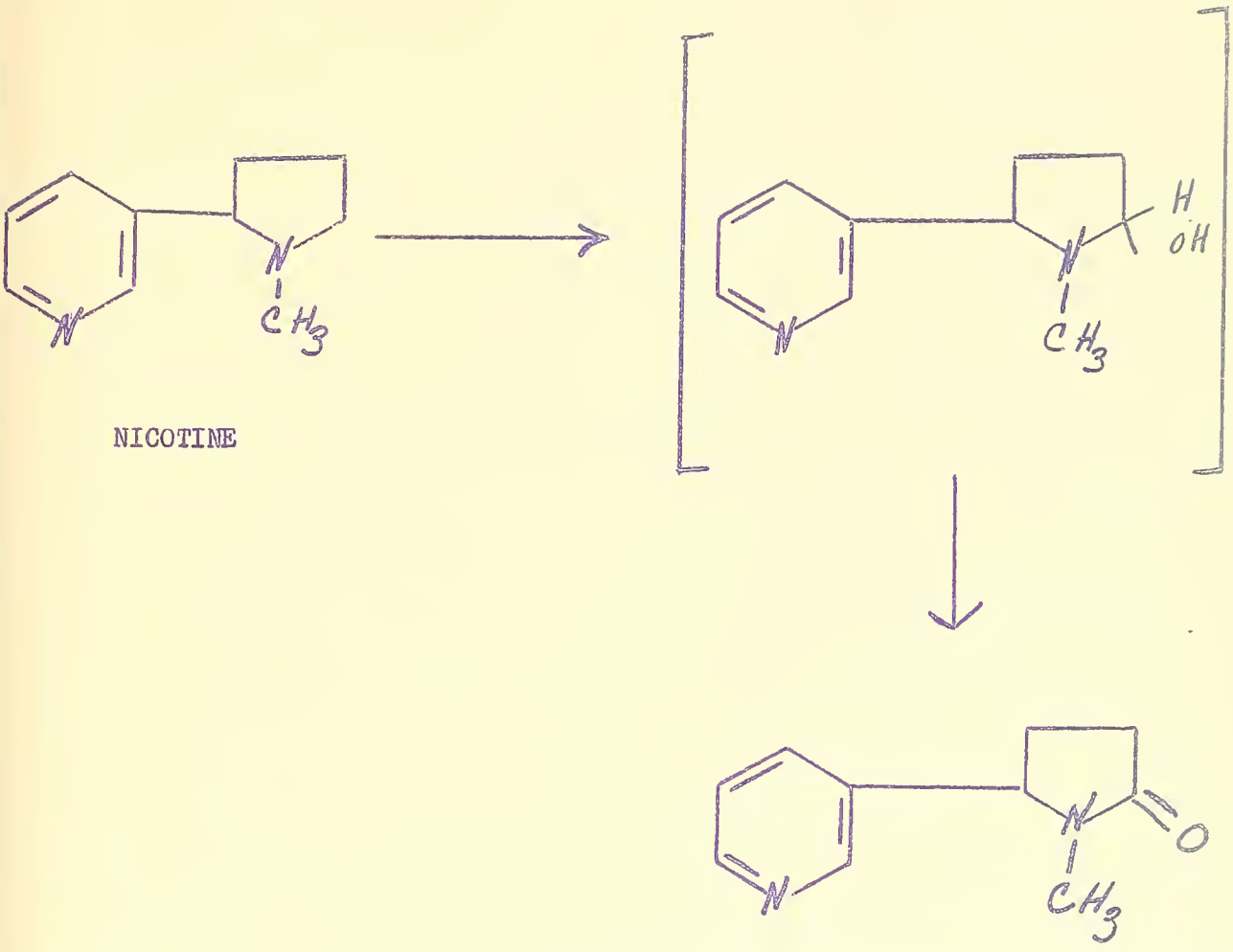
Approximately 10 per cent of the dose of nicotine administered to rabbits was shown to be excreted in the urine as cotinine. Only a minor fraction of the dose of cotinine administered was excreted unchanged. Therefore, it seems likely that cotinine is a major intermediate in the metabolism of nicotine in vivo.

Serial No. NHI-180

Significance to the Program of the Institute: The present study is contributing to the better understanding of the metabolic breakdown of nicotine, a drug of considerable pharmacological importance.

Proposed Course of Project: Of immediate interest is the attempt to identify the metabolic precursor of cotinine, which at present is thought to be an amino-aldehyde. Investigation of the further metabolism of cotinine is also planned.

Part B included: No



NICOTINE

COTININE

Serial No. NHI-181
1. Chemical Pharmacology
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Cardiac Hypersensitivity and Fatty Changes in the Myocardium

Principal Investigator: Dr. Harriet M. Maling

Other Investigator: Mrs. Martha A. Williams

Cooperating Unit: Dr. Benjamin Highman, National Institute of Arthritis and Metabolic Diseases

Man Years (calendar year 1958):
Total: 0.2
Professional: 0.1
Other: 0.1

Patient Days (calendar year 1958):
None

Project Description:

Objectives: To determine whether fatty changes in the myocardium and cardiac hypersensitivity are causually related. In this laboratory, two methods have been studied for producing in conscious dogs an abnormal state lasting days during which small test doses of either epinephrine or norepinephrine induce ventricular tachycardia: (1) In dogs with myocardial infarcts resulting from two-stage coronary occlusion, cardiac arrhythmias induced by test doses of catechol amines are exaggerated for approximately 12 days (Maling and Moran, Circulation Research, 5: 409, 1957). (2) A similar state of exaggerated drug-induced cardiac arrhythmias lasting 2-5 days occurs in dogs following an intravenous infusion of a large dose of norepinephrine or epinephrine (Maling and Highman, Am. J. Physiol. 194:590, 1958). Myocardial fatty changes are conspicuous in both of these abnormal states of cardiac hypersensitivity. During the period of hypersensitivity following coronary occlusions, marked fatty changes are demonstrable in the region surrounding the infarct. The myocardial fatty changes after infusion of catechol amines are widespread and patchy.

Serial No. NHI-181

In this project, we hope to answer the following questions: (1) Is cardiac hypersensitivity always present when myocardial fatty changes are present? (2) Are fatty changes invariably present when myocardial hypersensitivity is demonstrable?

Methods Employed: Electrocardiographic responses to test doses of norepinephrine are recorded before and at varying times after procedures which might be expected to cause fatty changes in the myocardium. The induction of ventricular tachycardias by these test doses indicates cardiac hypersensitivity.

Dogs are killed for pathologic examination by the intravenous administration of sodium pentobarbital. Tissues are fixed in 10% formalin buffered to pH 7.0. Frozen sections of the myocardium are stained for the presence of neutral fat with Oil red O.

Major Findings: The duration of fatty changes in the myocardium following catechol amine infusions in normal dogs and following coronary artery occlusion corresponds roughly to the duration of cardiac hypersensitivity.

Exposure of normal dogs to a simulated altitude of 38,000 ft. for 3.5 hours does not produce cardiac hypersensitivity of significant myocardial fatty changes, suggesting that hypoxia alone is not sufficient to produce these phenomena.

Diphtheria toxin does not cause myocardial fatty changes or cardiac hypersensitivity in the dog (six animals), even though cardiac arrhythmias and fatty changes in the heart have been reported in man after diphtheria. Marked jaundice is produced in the dog by diphtheria toxin.

Intravenous infusions of serotonin (in doses up to 1mg/kg) over a period of about 90 minutes either do not affect arterial pressure significantly or lower the pressure moderately. There is no cardiac hypersensitivity the next day. The monoamine oxidase inhibitor JB 516, given daily by subcutaneous injection in a dose of 2 mg/kg, does not cause cardiac hypersensitivity.

Mild cardiac hypersensitivity was demonstrated in one dog after chloroform inhalation. However, liver damage is more marked than cardiac damage after chloroform, and it is difficult to produce a prolonged reversible state of cardiac hyperexcitability with this agent. Most dogs either show no after-effects or die within 2-3 days.

Signficance to the Program of the Institute: This project may give insight into the physiological and biochemical mechanisms underlying prolonged states of cardiac hypersensitivity. An understanding of these mechanisms may provide a rational basis for the selection of

potential new antiarrhythmic drugs.

Proposed Course of Project: The search for additional methods of producing prolonged states of cardiac hypersensitivity will be continued. Pathologic studies will be made on dogs showing marked cardiac hypersensitivity.

Part B included: yes

Serial No. NHI-181

Publications:

Maling, H.M. and B. Highman. Exaggerated ventricular arrhythmias and myocardial fatty changes after large doses of norepinephrine and epinephrine in unanesthetized dogs. Am. J. Physiol. 194:590, 1958.

Maling, H.M. and B. Highman. High altitude tolerance of normal dogs and dogs with myocardial infarcts. Am. J. Physiol., in press.

Honors and Awards: None

Serial No. NHI-182
1. Chemical Pharmacology
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Studies on Antiarrhythmic Drugs

Principal Investigator: Dr. Harriet M. Maling

Investigator (Other): Mrs. Martha A. Williams

Cooperating Unit: None

Man Years (calendar year 1958):
Total: 0.2
Professional: 0.1
Other: 0.1

Patient Days (calendar year 1958):
None

Project Description:

Objectives: To test drugs for antiarrhythmic activity against the spontaneous ectopic activity which is conspicuous in unanesthetized dogs the day after ligation of the anterior descending coronary artery. To study other pharmacological actions of antiarrhythmic drugs.

Methods Employed: The anterior descending coronary artery of dogs is ligated by the two-stage occlusion procedure of Harris (Circulation 1: 1318, 1950). Electrocardiograms and arterial pressure are recorded the day after occlusion when spontaneous ectopic activity is most marked. The drugs being tested are injected intravenously in appropriate doses over a period of one minute. Observations are continued for a period of at least one hour after the drug.

Major Findings: The following compounds have been tested for antiarrhythmic activity during the past year: three alkaloids isolated by Dr. Wildman (lycoramine, belladine and crinine); two Squibb compounds somewhat related chemically to procaine amide (SQ 9737 and SQ 9738); and five Abbott compounds which are barbiturate derivatives with an amide side chain. The alkaloids isolated by Dr. Wildman does not possess antiarrhythmic activity. SQ 9738 may have moderate antiarrhythmic activity. All the members of the Abbott barbiturate series show at least some antiarrhythmic activity; these compounds are not anesthetics.

Serial No. NHI-182

Significance to the Program of the Institute: Testing selected drugs for antiarrhythmic activity in dogs may lead to the discovery of a clinically-useful antiarrhythmic drug.

Proposed Course of Project: It is planned to test additional members of the Abbott series of barbiturate derivatives. Promising members of this series will be tested further for toxicity and other pharmacological actions. Our data will be combined with data obtained by Dr. Schmidt and prepared for publication.

Part B included: No

Serial No. NHI-183
1. Chemical Pharmacology
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Drug-induced Ventricular Arrhythmias before and after Coronary Artery Occlusion

Principal Investigator: Dr. Harriet Maling

Other Investigator: Mrs. Martha A. Williams

Cooperating Unit: None

Man Years (calendar year 1958):
Total: 0.2
Professional: 0.1
Other: 0.1

Patient Days (calendar year 1958):
None

Project Description:

Objectives: To compare various sympathomimetic drugs with respect to their effects upon the spontaneous ectopic activity after coronary artery occlusion and their electrocardiographic responses during the period of cardiac hypersensitivity following occlusion (Maling and Moran, Circulation Research 5:409, 1957).

To determine whether the cardiac hypersensitivity after occlusion is restricted to sympathomimetics or is demonstrable with any cardiac stimulant drug. Arrhythmias induced by ouabain are given special attention.

Methods Employed: Electrocardiographic responses to test doses of various drugs are recorded before and at varying times after ligation of the anterior descending coronary artery in dogs, using the two-stage occlusion procedure of Harris (Circulation, 1:1318, 1950). Usually, responses are compared on the first and fourth days after occlusion.

Major Findings: After coronary artery occlusion, the heart is hypersensitive to ouabain as well as epinephrine and norepinephrine. Doses of ouabain which do not produce ectopic beats in normal dogs cause considerable ectopic activity and sometimes ventricular tachycardia after occlusion, even on the fourth day when spontaneous ectopic activity is absent.

Serial No. NHI-183

Sympathomimetic drugs vary considerably in their tendency to produce ectopic activity on the fourth day after coronary artery occlusion and in their effects on the spontaneous ectopic activity. These effects seem to be correlated with their effects upon contractile force of the heart.

Significance to the Program of the Institute: This study may be helpful in selecting drugs for use after coronary occlusion in man.

Proposed Course of Project: The comparisons of arrhythmias induced by sympathomimetics before and after coronary artery occlusion will be continued. The study will be extended to include other cardiac stimulant drugs - including serotonin and theophylline.

Part B included: No

Serial No. NHI-184
1. Chemical Pharmacology
2. Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Studies on the Pharmacology of JB 516 and other Inhibitors of Monoamine Oxidase

Principal Investigators: Dr. Harriet M. Maling
Dr. Sydney Spector

Other Investigators: Mrs. Martha A. Williams
Mr. William M. Butler, Jr.

Cooperating Unit: Dr. Benjamin Highman, NIAMD

Man Years (calendar year 1958):
Total: 0.5
Professional: 0.3
Other: 0.2

Patient Days (calendar year 1958):
None

Project Description:

Objectives: To study the pharmacological actions of JB 516 and other monoamine oxidase inhibitors. To correlate pharmacological actions, levels of serotonin, norepinephrine and dopamine in various parts of the central nervous system, and pathologic findings, if possible. JB 516 merits special study because clinical tests of this compound seem promising.

Methods Employed: Observations are made on unanesthetized animals. In some dogs and rabbits, electrocardiograms, arterial pressure and the response to tilt are recorded before and at varying times during the course of daily subcutaneous doses of the inhibitor. In some animals, the responses to test doses of norepinephrine have also been studied. Hematocrits, blood hemoglobin values, and serum transaminase and alkaline phosphatase levels have been measured before and at varying times during the course of daily doses.

Animals are killed with sodium pentobarbital. The norepinephrine and serotonin concentrations in the brain stem have been measured in some dogs and rabbits. Tissues for pathologic studies have been fixed in appropriate solutions.

Serial No. NHI-184

Major Findings: In dogs given daily doses of 2 mg/kg JB 516, the serotonin concentration of the brain stem increases markedly and the norepinephrine concentration remains unchanged. In contrast, in rabbits maintained on the same dose, both the norepinephrine and epinephrine concentrations in the brain stem increase.

Dogs did not show any significant change in arterial pressure when maintained on 2 or 4 mg/kg JB 516 (in some dogs, 5 days/week) for periods of 4-20 days. Even when neurological symptoms were marked, these dogs maintained their resting arterial pressure during tilting.

Rabbits given 2 mg/kg JB 516 for 4 days (1 rabbit) and 10 days (1 rabbit) did not show any change in arterial pressure and did not show postural hypotension during tilting.

Three of six dogs given 2 mg/kg JB 516 daily for 4-19 days showed symptoms of ataxia. These dogs were unsteady when standing. The hind legs tended to sink towards the floor.

All of 12 dogs maintained on 4 mg/kg JB 516 daily have developed marked neurological symptoms involving especially the legs. Five of these dogs have been given pyridoxine (5 mg/kg intramuscularly) daily, beginning with the first day of treatment with JB 516. Since neurological symptoms are prominent even in dogs given pyridoxine daily, the toxicity of JB 516 in dogs is not due primarily to pyridoxine deficiency.

Neurological findingsinclude general irritability, pupillary dilation and sluggish light reflex, unsteadiness during standing, sinking of the hind legs, rigidity of the hind legs, extensor spasms especially in the front legs, tremor which is most marked during rest, nystagmus (2 dogs) priapism (2 dogs), and changes in the character of the barking. Despite a good appetite, some dogs lose weight when given 4 mg/kg daily.

Neurological symptoms are evident after the second or third daily doseof 4 mg/kg JB 516. One dog died during the night after the third dose. Hematocrit and hemoglobin values were not changed significantly during the first week at this dose, but fell appreciably during the second week. Serum transaminase and alkaline phosphatase values may rise moderately.

Significance to the Program of the Institute: The toxicity of JB 516 is especially interesting since this drug has been given a clinical trial in the Institute.

Proposed Course of Project: Careful histological studies will be made of the spinal cord and brain of dogs maintained on JB 516, 4 mg/kg daily. The study will be extended to other monoamine oxidase inhibitors.

Part B included: No.

Serial No. NHI-185
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: A Search for Physiologically Active, Fluorescent Compounds in Animal Tissues

Principal Investigator: Dr. J.A.R. Mead

Other Investigator: None

Cooperating Unit: None

Man Years (calendar year 1958):
Total: 1
Professional: 1
Other: 0

Patient Days (calendar year 1958): None

Project Description:

Objectives: In the past few years relatively high concentrations of the physiologically active amines, serotonin, norepinephrine and dopamine, have been found in animal tissues. They seem to be concentrated more especially in the central autonomic nervous system and their presence in this system has led to the development of hypotheses involving the physiological mechanisms of the brain. There are, however, areas in the body where the above-mentioned amines are found in comparatively low concentrations, e.g., in some regions of the brain, and this prompted us to consider the possibility that in these areas other physiologically active compounds may be present. Although the brain was considered in the first instance, other tissues were not excluded from consideration especially those which exhibit marked physiological changes under the influence of drugs or pathological conditions.

An essential feature of any method adopted was considered to be its sensitivity as we did not anticipate dealing with amounts of material in excess of 1 μg/g of tissue. To achieve sensitivities of this order we relied to a great extent on spectrophotofluorometry, a technique already proved of value in this field of investigation.

Serial No. NHI-185

Methods Employed: Established methods.

Major Findings: Using a procedure, developed in this laboratory for the extraction of norepinephrine from tissues, several rabbit tissues were examined. In each case the results were inconclusive, the only fluorescent compound positively identified being serotonin.

At this stage, a report in the literature on the presence of an unidentified vasoactive substance in the nasal mucosa of dogs and sheep led to an examination of the nasal mucosa of dogs. It was discovered that there is present in mucosal extracts a definite fluorophoric substance, which behaves as a single compound on paper chromatograms and counter-current distribution. Its extraction properties are those of a neutral compound, and on paper chromatograms it gives none of the common color reactions. Also, it has no biological activity when tested against the isolated guinea pig ileum, a fact which distinguishes it from the substance reported above.

The fluorophore seems to be concentrated in the nasal mucosa and up to the present has not been detected elsewhere in the other tissues examined including other mucosal tissues.

An incidental finding of significance was that serotonin was quantitatively extracted by the method developed for norepinephrine and this led to the development of a combined method for the two amines, using the one extraction procedure instead of the two which had been developed previously. This will facilitate research programs which involve the quantitative assay of norepinephrine and serotonin.

Significance to the Program of the Institute: The characterization and identification of new physiologically active, organ specific substances will lead to a clearer understanding of both the normal and pathological processes which take place in the animal body.

Proposed Course of Project: Further investigation into the properties, distribution and biological activity of the mucosal fluorophore will be carried out.

Part B included: No

Serial No. NHI-186
1. Chemical Pharmacology
2. Drug Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Studies in Biochemical Evolution
V. The ontogenetic development
of the microsomal drug enzymes

Principal Investigators: Mr. Roger P. Maickel
Dr. Werner Robert Jondorf

Other Investigator: None

Cooperating Unit: None

Man Years (calendar year 1958):
Total: 0.3
Professional: 0.1
Other: 0.2

Patient Days (calendar year 1958):
None

Project Description:

Objectives: Studies with the tadpoles of terrestrial amphibia such as toads and some frogs and the juvenile aquatic stage of terrestrial salamanders showed that these immature animals lacked the liver enzymes for metabolizing foreign compounds. In contrast to this, the adult of the species did have the enzymes. These results suggested that a parallel course of "appearance" of the enzymes might be found in the development from aquatic to terrestrial life which occurs in the transition from foetal to postnatal life in mammals.

Methods Employed: Standard *in vivo* and *in vitro* methods for studying the metabolism of drugs were used.

Major Findings: Preliminary results in guinea pigs and mice indicate that the microsomal drug enzymes responsible for N-dealkylation, O-dealkylation, sidechain oxidation, and glucuronide formation are not present in the foetus, but rather develop at about 4 to 10 days after birth. This is in agreement with the concept "ontogeny recapitulates phylogeny" since the fetal animals in its aquatic environment has no need for duplication of the enzymatic mechanisms available to it in the maternal livers. Transfer of lipid soluble substances across the

placental membrane may thus be likened to similar transfer across the lipid membrane of the fish gill.

Significance to the Program of the Institute: These studies suggest that the microsomal enzymes responsible for the oxidative metabolism of drugs are furth r examples of "ontogeny recapitulating phylogeny". The practical significance of these studies is in the field of therapeutics. The lack of these enzymes in the newborn indicates that drugs should be used in the newborn with extreme caution and may explain the toxic effects to the newborn child of some/drugs given to the mother prior to delivery.

Proposed Course of Project: Further studies are necessary in order to ascertain more clearly the time of development of all enzymes, for the metabolism of foreign compounds including those for 1) sulfur oxidation, 2) mercapturic acid, 3) ester and amide hydrolysis, and 4) dehalogenation. It will also be of interest to study the mechanisms which control their development and the possible role of hormones in this control.

Part B included: No

Serial No. NHI-187
1. Chemical Pharmacology
2. Drug Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Studies in Biochemical Evolution
IV. The metabolism of drugs and foreign organic compounds by arthropods

Principal Investigators: Dr. Werner Robert Jondorf
Mr. Roger P. Maickel

Other Investigator: None

Cooperating Unit: None

Man Years (calendar year 1958):
Total: 0.7
Professional: 0.6
Other: 0.1

Patient Days (calendar year 1958):
None

Project Description:

Objectives: The development of enzymes responsible for the metabolism of lipid soluble foreign organic compounds may have taken place at a specific point in the evolutionary development of a given phyla. Mammals and the higher orders of vertebrates possess enzymes capable of carrying out a variety of oxidative reactions on lipid soluble foreign compounds. In connection with this, it is of interest to study the enzymes in arthropods, which were developed presumably for the same purpose.

Methods Employed: Standard *in vivo* and *in vitro* methods for the metabolism of drugs were used.

Major Findings: Insects such as house crickets and lubber grasshoppers showed a great facility for metabolizing drugs which are oxidized in mammals by microsomal enzymes. A homogenate of the gastrointestinal tract of the lubber grasshopper showed enzymatic activity when incubated

Serial No. NHI-187

with aniline, monomethyl-4-aminoantipyrine or 4-aminoantipyrine. Evidence was found for an acid labile metabolite of aniline, involving the amino group. No cofactor requirements have been discovered as yet.

In a study of aquatic arthropods such as crayfish and lobsters, compounds such as aminopyrine and chlorpromazine were metabolized in vivo. Attempts to locate the site of enzymatic activity by in vitro studies using the hepato-pancreas were unsuccessful asthe preparations did not respond to attempts at activation with a broad spectrum of mammalian cofactors.

Significance to the Program of the Institute: A study of this type is important in the production of data which could produce a significant biochemical differentiation of species.

Proposed Course of Project: Attempts will be made to determine the specific localization of these enzymes in the arthropods. At the time there are indications that the cofactors involved may be very much different from those utilized by similar enzyme systems in mammals. The mechanisms of metabolism of foreign compounds in arthropods will be studied.

Part B included: No

Serial No. NHI-188
1. Chemical Pharmacology
2. Drug Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Studies in Biochemical Evolution
III. The metabolism of foreign compounds by toads

Principal Investigator: Mr. Roger P. Maickel

Other Investigator: None

Cooperating Unit: None

Man Years (calendar year 1958):
Total: 0.3
Professional:
Other: 0.3

Patient Days (calendar year 1958):
None

Project Description:

Objectives: The toad is an amphibian which spends its adult life on dry land. It lacks the damp, semi-permeable skin of the frog and thus must dispose of non-polar foreign compounds by metabolism to products which can be excreted by the kidney. Preliminary findings with Bufo marinus, selected because of completely terrestrial life, suggested that the toad metabolizes many foreign organic compounds by other mechanisms. Since these systems developed independently of those in reptiles their study afforted an opportunity to see whether nature solved the problem by different or the same mechanisms. This is a fundamental question in biochemical evolution.

Methods Employed: Standard in vivo and in vitro techniques for drug metabolism were used.

Major Findings: Studies with two other species, Bufo fowleri and Bufo americanus confirmed the mechanisms of oxidation for drugs and

foreign compounds in toads was indeed different from those in mammals. The enzymes involved were found to be localized in the soluble fraction rather than in the microsomes, and did not require reduced triphosphopyridine nucleotide (TPNH) and oxygen. Further, inhibitors of the mammalian enzymes, such as SKF 525-A were without effect on the toad enzyme systems. Properties of enzymes indicates that dehydrogenase are involved - whereas in reptiles, birds and mammals the enzymes activate oxygen from the air.

A detailed study of the metabolism of MMAP and aminopyrine in B. marinus has shown these drugs to be metabolized along a different pathway than in mammals. MMAP is not demethylated but is rather converted to 4-hydroxyantipyrine. On the other hand, aminopyrine is demethylated to MMAP, but by dehydrogenation rather than an oxidative mechanism. The enzyme loses activity on dialysis and may be reactivated by addition of TPN.

Significance to the Program of the Institute: The importance of this problem to the evolutionary development of animals lies in the question of whether a biochemical function which is solved more than once in evolution will be solved differently in each instance.

Proposed Course of Project: Further studies on the enzymes involved will be carried out. Particular emphasis will be placed on the mechanisms involved in these processes, the co-factors involved, and the products formed. Attempts will be made to isolate and purify the enzymes involved.

Part B: None

Serial No. NHI-189
1. Chemical Pharmacology
2. Drug Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Studies in Biochemical Evolution
II. Glucuronide and ethereal sulfate conjugating mechanisms

Principal Investigators: Mr. Roger P. Maickel
Dr. Werner Robert Jondorf

Other Investigator: None

Cooperating Unit: A portion of this work is being carried out in collaboration with Dr. Vernon C. Applegate of the U.S. Fish and Wildlife Service, Rogers City, Michigan

Man Years (calendar year 1958):
Total: 0.3
Professional: 0.1
Other: 0.2

Patient Days (calendar year 1958):
None

Project Description:

Objectives: A number of foreign phenols are intrinsically toxic to the animal organism. If lipid soluble, phenols are not readily excreted by the kidney, but higher vertebrates possess mechanisms that convert these phenols to glucuronides or ethereal sulfates which are readily excretable. These mechanisms are present in mammals, birds, reptiles and amphibia, but are absent in fish which are extremely sensitive to the effects of foreign phenolic compounds. This project is a study of the evolutionary development of the conjugative reactions.

Methods Employed: Standard in vivo and in vitro techniques for the metabolism of foreign phenols were used.

Serial No. NHI-189

Major Findings: Amphibia such as frogs and salamanders were found to have glucuronide conjugating mechanisms similar to those of higher vertebrates. However, all attempts to produce phenyl glucuronides in fish, in vivo, or in liver slices or homogenates reinforced with glucose, were unsuccessful. A study was made of the missing factor in fish. Surprisingly enough fish microsomes have the glucuronide transferase. Thus incubation with phenolphthalein and exogenous UDPGA (active glucuronic acid) resulted in formation of phenolphalein glucuronide. However, the soluble fraction of fish liver (78,000 x g supernatant) was found to be lacking in the DPN dependent dehydrogenase enzyme which is required for the oxidation of uridine diphosphoglucose to UDPGA.

Significance to the Program of the Institute: The development of the mechanisms for glucuronide and sulfate conjugation are exceedingly important in the metabolism of foreign phenols, alcohols, acids and amines, and also in the excretion of normal body constituents such as sterols and bilirubin. The reason for their development in evolution would be a great help in understanding their function.

Proposed Course of Project: Further studies will be carried out to more specifically describe the enzymatic defect in glucuronide formation in the fish. The mechanism of sulfate conjugation will be studied in a similar manner. An attempt will be made to determine the effects of lack of these mechanisms on the metabolism and excretion of bilirubin and sterols in the fish. Animals forms both aquatic and terrestrial which are lower than fish in evolutionary scale will be studied to see how they dispose of phenols and to obtain an answer as to whether the enzymes are in intermediary metabolism important.

Part B included: No

Serial No. NHI-190
1. Chemical Pharmacology
2. Drug Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Studies in Biochemical Evolution
I. General Concepts

Principal Investigators: Mr. Roger P. Maickel
Dr. Werner Robert Jondorf

Other Investigator: None

Cooperating Unit: None

Man Years (calendar year 1958):
Total: 0.4
Professional: 0.2
Other: 0.2

Patient Days (calendar year 1958):
None

Project Description:

Objectives: There are many instances in the evolutionary scale in which nature was faced with a problem that had to be solved if development was going to continue. This is illustrated quite well by the development of lungs in the amphibia when they left the aquatic environment of their predecessors. The inability of kidneys to secrete lipid soluble compounds suggests that with the escape from water, there also developed special systems for making fat-soluble substances ingested in food (and which are often structural prototypes of drugs) less lipid soluble. These experiments describe how this problem was solved in various phyla and represent an approach to studying how enzymes responsible for function may have developed.

Methods Employed: Standard in vivo and in vitro techniques for studying the metabolism of drugs were used.

Major Findings: (1) Oxidation of Foreign Compounds: A variety of oxidative enzymatic mechanisms for the metabolism of drugs were studied in various lower phyla. In general, they are present in the terrestrial vertebrates. Thus, in a study of enzymatic mechanisms for

the oxidation of barbiturates, N-alkylamines, aromatic ethers, primary amines and aromatic rings, fish were found to be totally lacking the enzymes. Amphibia are the crossroads of the evolutionary pathway from aquatic to terrestrial existence.

It was found that those leading an aquatic life lack the enzymes, while those amphibia which maintain a terrestrial existence possess the oxidative mechanisms. The reptiles possess oxidative mechanisms some of which are localized in liver microsomes, similar to those of the mammals, while others are localized in the soluble fraction of the liver.

A similar study of arthropods showed both insects and crustaceans to be able to oxidize foreign compounds, the fully terrestrial insects much more efficiently than partly terrestrial arthropods.

(2) Other enzymes for metabolism of foreign compounds: A similar phylogenetic study of the enzymatic mechanisms for conjugation of foreign phenols has led to the discovery that these pathways are absent in fish but present in other vertebrates. Other workers have shown insects to form glycosides of foreign phenols.

These results suggest that the development of enzymatic mechanisms for the metabolism of many foreign organic compounds in animals parallel the change in the animals' environment, i.e. from aquatic to terrestrial. As aquatic creatures they did not need these mechanisms since they were able to excrete lipid soluble foreign compounds through lipoidal gills or skins. With emergence onto land, however, there arose a need for conservation of water. This was solved with a less permeable skin, which necessitated the development of mechanisms to cope with lipid soluble foreign compounds.

Significance to the Program of the Institute: This work explores the way in which various animals have solved biochemical problems involved in their evolutionary development. It thus represents an approach toward an understanding of the mechanisms involved in the biochemistry of function.

Proposed Course of Project: Other pathways for the metabolism of foreign compounds will be studied to see if they fit the concept of "water conservation" or if they are in reality necessary parts of intermediary metabolism.

Part B included: Yes

Serial No. NHI-190

Part B

Honors, Awards and Publications: Annual Lakeside Lecture - Chicago Annual Smith-Reed-Russel Society Lecture - Georg. Washington University Medical School.

Publications: Termination of drug action by metabolic inactivation. B.B.Brodie, R.P. Maickel, W.R. Jondorf. Fed. Proc., in press.

Serial No. NHI-191
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Studies on Mechanism of Action of Ergotropic Agents

Principal Investigator: Dr. D.F. Bogdanski (6 months)

Other Investigators: Dr. Fridolin Sulser
Mr. James Watts

Cooperating Unit: Dr. Sulser on Fellowshio from the Swiss Academy of Medical Science

Man Years (calendar year 1958):
Total: 3/4
Professional: 1/4
Other: 1/2

Patient Days (calendar year 1958):
None

Project Description:

Objectives: Ergotropic agents produce an excitatory pattern involving behavior, the somatic system and the sympathetic nervous system. These agents show structural similarities to norepinephrine which is postulated as the chemical intermediary of the ergotropic system of Hess. The purposes of the present investigation are: To demonstrate a central site of action in relation to autonomic as well as behavioral effects and to employ the ergotropic agents as tools for the study of central autonomic depressants such as chlorpromazine and reserpine.

Methods Employed: Usual procedures used in pharmacology.

Major Findings: (1) All lipid soluble congeners of norepinephrine, as well as cocaine, gives typical ergotropic effects including excitement, EEG arousal pattern, increased responsiveness to external stimuli, increased psychomotor activity and increased central sympathetic outflow. Since DOPA gives the same responses it is probable that the ergotropic system is an adrenergic system and may be synonymous with the teticuloactivating system. (2) Some ergotropic agents stimulate peripheral adrenergic receptors as well as central sites, thus an effect of amphetamine on blood pressure is seen even following spinal section.

Serial No. NHI-191

(3) Effects of ergotropic agents are blocked by barbiturate anesthesia. Thus, the hypertension and tachycardia elicited by ergotropic agents may even be reversed following anesthesia. (Many effects of drugs on central sites have been missed by others who have worked with anesthetized animals). (4) Results with LSD are now in accord with the view that it acts centrally not by blocking serotonin, but by mimicking norepinephrine. (5) Preliminary results indicate that LSD and cocaine compete in brain for some receptors as chlorpromazine.

Significance to the Program of the Institute: Norepinephrine and other amines in brain are involved in control of cardiovascular integrations.

Proposed Course of Research: The central actions of the ergotropic agents will be investigated more thoroughly and coupled to the normal distribution of norepinephrine and its postulated opposite, serotonin. Neurophysiological techniques will be employed to study central effects directly. The antagonisms between the ergotropic agents and central autonomic inhibitors are being investigated to demonstrate differences in the mechanisms of action of the central depressants.

Part B included: No

Serial No. NHI-192
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Pharmacologic Mechanism of Reserpine Action in Brain

Principal Investigators: Dr. D. Bogdanski (6 months)
Dr. Fridolin Sulser

Cooperating Unit: Dr. Sulser on Fellowship from the Swiss Academy of Medical Science

Man Years (calendar year 1958):
Total: 1/2
Professional: 1/2
Other: 0

Patient Days (calendar year 1958)
None

Project Description:

Objectives: Reserpine has been postulated to act centrally by stimulating through serotonin, a neuronal system (trophotropic) which integrates the parasympathetic system with somatomotor and psychic functions. This system seems to act in opposition to a system (ergotropic) which integrates the sympathetic system with somatomotor and psychic functions. This latter system is an adrenergic system which is antagonized by chlorpromazine.

If this conception is valid, then reserpine, in contrast to chlorpromazine, should increase the parasympathetic output from the central nervous system.

Methods Employed: Usual methods of classical pharmacology.

Major Findings: (1) The apparent decrease in central sympathetic output following reserpine is not central in origin. It has been satisfactorily explained by the depletion of norepinephrine at peripheral nerve endings. (2) Pupillary constriction induced by reserpine in rabbits and cats is entirely due to central parasympathetic stimulation. (3) Lacrimation produced by reserpine in rabbits and rats is a central parasympathetic response. (4) The enhanced light reflex is a parasympathetic action of central origin.

(5) The signs elicited by chlorpromazine are in accord with the view that it stimulates a "sleep" center while chlorpromazine suppresses an arousal mechanism. Thus reserpine produces active closure of eyelids and extreme miosis as in sleep or stimulation of trophotropic system; chlorpromazine produces relaxation of eyelids (ptosis) and partial miosis similar to that obtained by antagonizing the central arousal mechanisms.

Significance to the Program of the Institute: An understanding of action of reserpine will lead to understanding of function of norepinephrine and serotonin in various brain functions.

Proposed Course of Project: To ascertain whether reserpine stimulates other parasympathetic functions such as gastric secretion, nasal secretion, salivation, etc.

Part B included: yes

Serial No. NHI-192

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Brodie, B. B., Spector, S., and Shore, P. A.: Interaction of Drugs with Norepinephrine in the Brain. Pharmacological Reviews, in press.

Shore, P. A.: A Simple Technique Involving Solvent Extraction for the Estimation of Norepinephrine and Epinephrine in Tissues. Pharmacological Reviews, in press.

Brodie, B. B.: Interaction of Psychotropic Drugs with Physiologic and Biochemical Mechanisms in Brain. Modern Medicine, August 1, 1958, pp. 69-80.

Udenfriend, S. Weissbach, H., and Brodie, B. B.: Assay of Serotonin and Related Metabolites, Enzymes and Drugs. Methods of Biochem. Analysis 6, 96, 1958.

Brodie, B. B., Prockop, D. J., and Shore, P. A.: An Interpretation of the Action of Psychotropic Drugs. Postgraduate Medicine, 24, 1958.

Brodie, B. B., and Shore, P. A.: On the Mechanism of Action of Psychotropic Agents. Book consisting of proceedings on the symposia on the Pharmacological Treatment of Schizophrenics given at the Second International Congress of Psychiatry in Zurich, Switzerland, 1957.

Brodie, B. B., Bogdanski, D. F., and Shore, P. A.: Biochemical and Physiological Interpretation of the Action of Psychotropic Drugs. In book entitled "Chemical Concepts of Psychosis," McDowell, Obolensky, Inc., New York, 1958.

Shore, P. A., and Brodie, B. B.: Influence of Various Drugs on Serotonin and Norepinephrine in Brain. Chapter in book on Psychotropic Drugs, Elsevier Press, 1958.

Honors and Awards relating to this project: None

Serial No. NHI-193
1. Chemical Pharmacology
2. Drug Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: The Metabolism of Sulfur Compounds

Principal Investigator: Dr. James R. Gillette

Other Investigator: Mr. Jerome J. Kamm

Cooperating Units: None

Man Years (Calendar year 1958): Patient Days: None
Total: 1.1
Professional: .1
Other: .1

Project Description:

Objectives: To study the metabolism of sulfur compounds.

Methods Employed: A method based upon the differential extraction of 4,4'-diaminodiphenyl sulfoxide with methyl isobutyl ketone has been developed.

Major Findings: Guinea pig microsomes are capable of oxidizing chlorpromazine and 4,4'-diaminodiphenyl sulfide to chlorpromazine sulfoxide and 4,4'-diaminodiphenyl sulfoxide respectively. The enzyme system which carries out this oxidation has been shown to require reduced triphosphopyridine nucleotide and oxygen. A number of compounds such as p-chloromercuribenzoate, methylene blue and iodoacetate have been found to inhibit enzyme activity. On the other hand, SKF 525-A (β-diethylaminoethyldiphenylpropylacetate hydrochloride), a potent inhibitor of a number of microsomal drug enzymes, did not inhibit sulfoxidation.

Serial No. NHI-193

Gillette et al. have shown that TPNH is oxidized by liver microsomes to yield hydrogen peroxide. It has been possible to demonstrate a stoichiometric decrease in the amount of hydrogen peroxide formed in the presence of 4,4'-diaminodiphenyl sulfide although there is no associated decrease in the amount of TPNH oxidized. Based upon these findings, the following mechanism is proposed for sulfoxidation:

1) TPNH + O_2 + enzyme ——————→ (active enzyme-oxygen complex) + TPN

2) a. (Active enzyme-oxygen complex) ——→ H_2O_2 + enzyme

or

b. (Active enzyme-oxygen complex) + substrate ——→ substrate + enzyme

Significance to the Program of the Institute: As a result of these studies a better understanding of the enzymatic oxidation of sulfur compounds has been obtained.

Proposed Course of Project: Studies on sulfur metabolism will be continued. Emphasis will be placed upon the reduction of sulfoxides and sulfones.

Part B included: No

Serial No. NHI-194
1. Chemical Pharmacology
2. Drug Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: The Metabolism of Toluene and Other Alkyl Hydrocarbon Sidechains by Mammalian Liver Preparations

Principal Investigator: Dr. James R. Gillette

Other Investigator: Mr. James V. Dingell

Cooperating Units: None

Man Years (Calendar Year 1958): Patient Days: None
Total: .7
Professional: .1
Other: .6

Project Description:

Objectives: It has long been known that animals can oxidize alkyl hydrocarbon sidechains. However, little is known of this biotransformation at the enzyme level. Since a number of foreign compounds can be metabolized by the microsomal fraction of liver, it is of interest to determine whether the sidechain of toluene and other alkyl hydrocarbons such as p-n-butyl phenol are oxidized by the microsomal system.

Methods Employed: A method has been developed for the determination of p-n-butyl phenol. The procedure involves the differential extraction of the material into heptane at acid pH, washing of the organic phase with sodium carbonate and recovery of the phenol into alkali. Following neutralization, a color is developed by utilizing the reaction of the phenolic group with Folin-Ciocalteu reagent.

Major Findings: It has been previously reported that p-nitrotoluene is metabolized to p-nitrobenzyl alcohol by a TPNH dependent enzyme system localized in rabbit liver microsomes. p-Nitrobenzyl alcohol is converted to p-nitrobenzoic acid by a DPN dependent enzyme system in the liver soluble fraction.

Serial No. NHI-194

Williams et al. (Biochemical J. 64, 50-56, 1956) have postulated -oxidation to γ-phenyl butyric acid as one of the pathways in the in vivo metabolism of n-butyl benzene. However, the role of such a pathway remains, as yet, unproven.

Recent studies, in this laboratory, have demonstrated the disappearance of p-n-butyl phenol in a system requiring microsomes, oxygen and a TPNH generating system.

Significance to the Program of the Institute: The continuation of this program will provide a further understanding of the metabolism of a number of drugs.

Proposed Course of Project: The continuation of this project will necessitate the isolation and identification of metabolites as well as the characterization of the enzymes involved.

Part B included: yes

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Gillette, J. R. The Sidechain Oxidation of Alkyl Substituted Ring Compounds. I. Enzymatic Oxidation of p-Nitro Toluene. J. Biol. Chem., Jan. 1959.

Honors and Awards Relating to this Project: None

Serial No. NHI-195
1. Chemical Pharmacology
2. Drug Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Model Enzyme Systems in the Study of Drug Metabolism

Principal Investigator: Dr. James R. Gillette

Other Investigator: Mr. James V. Dingell

Cooperating Unit: None

Man Years (calendar year 1958):
Total: .7
Professional: .3
Other: .4

Patient Days (calendar year 1958): None

Project Description:

Objectives: A number of foreign compounds are oxidized by enzyme systems which are localized in the microsomal fraction of mammalian liver. However, the mechanism involved in these reactions is not clearly understood. The present studies were undertaken to utilize model systems to determine possible mechanisms for the microsomal reactions.

Methods Employed: Established methods.

Major Findings:
A. Dealkylation: As previously reported, the dealkylation of the methyl derivatives of aniline and 4-aminoantipyrine has been demonstrated in model systems which function through peroxidase, and oxidase and dehydrogenase mechanisms.

B. Sulfoxidation: Chlorpromazine can be converted to its sulfoxide by model systems through peroxidase or dehydrogenase mechanisms. Both of these reactions involve the formation of a red colored intermediate.

Serial No. NHI-195

Recently Dusinsky and Liskova (Chemicke Zvesti XII, 4, 213-20, 1958), haveshown that a red colored compound is formed when chlorpromazine is treated with an equivalent amount of ceric sulfate and have suggested that the red product may be a semi-quinoidinic free radical. We have confirmed this work and have shown that the reaction of chlorpromazine with excess ceric sulfate immediately yields a sulfoxide-like product. Furthermore, the red colored intermediate from the ceric sulfate reaction has an absorption spectrum identical to that from the ferric chloride and horseradish peroxidase system.

C. Replacement of Sulfur by Oxygen: Winters et al. (J. Pharmacol. Exp. Therap., 114, 343, 1955) have shown that thiopental is converted to pentobarbital and inorganic sulfate with rat liver preparations by an unknown pathway. In this light, it is interesting that 0.05% hydrogen peroxide solutions (pH 7.4) oxidize thiopental to a compound with absorption maxima at 356 and 252 (pH 7.4) or 356, 282, 246 (pH 10.0). After treatment with catalase to remove excess perioxide, this compound is stable for several days at pH 7.4, but it rapidly decomposes to pentobarbital on acidification.

Significance to the Program of the Institute: Studies with model systems may be utilized to determine possible mechanisms for the microsomal reactions.

Proposed Course of Project: The identity and chemical properties of the oxidized derivative of thiopental and its possible role in biological systems will be studied. Further studies will be devoted to determining the capabilities of other model systems, such as the dihydroxy furmaratehorseradish peroxidase system of Mason et al. (Biochim. et Biophys. Acta 24, 225, 1957).

Part B included: Yes

Serial NO. NHI-195

Publications:

Gillette, J.R., Dingal, J.V., and Brodie, B.B.; Dealkylation of N-alkylamines by Model Systems, Nature 181, 898, 1958.

Honors and Awards: None

Serial No. NHI-196
1. Chemical Pharmacology
2. Drug Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: The Metabolism of Alcohols and Aldehydes

Principal Investigator: Dr. James R. Gillette

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1958): Patient Days: None
Total: .5
Professional: .5
Other: 0

Project Description:

Objectives: The function of liver alcohol dehydrogenase and liver aldehyde dehydrogenase in the metabolism of ethanol is well known. However, surprisingly little is known of the role of these enzymes in the metabolism of the higher alcohols.

Major Findings: p-Nitrobenzyl alcohol is oxidized to p-nitro benzoic acid by crystalline liver alcohol dehydrogenase and crude kidney aldehyde dehydrogenase although at a very slow rate.

Approximate values for the Michaelis constants of crystalline liver alcohol dehydrogenase were determined with a number of alcohols at pH 9.3. These results suggest that the affinity of liver alcohol dehydrogenase for n-propanol, n-butanol and isoamyl is greater than for ethanol. Furthermore, very small concentrations of p-nitrobenzyl alcohol (3×10^{-5}M) were found to inhibit the oxidation of ethanol from 56 per cent to 78 per cent. The data were not precise enough to determine whether this inhibition was competitive or non-competitive.

Significance to the Program of the Institute: The results of this study suggest that liver alcohol dehydrogenase and aldehyde dehydrogenase may be involved in the metabolism of a number of drugs.

Proposed Course of Project: The metabolism of a number of other alcohols and aldehydes will be investigated.

Part B included: yes

Serial No. NHI-196

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Gillette, James R. Sidechain oxidation of alkylsubstituted ring compounds. I. Enzymatic oxidation of p-nitrotoluene. J. Biol. Chem., Jan. 1959.

Brodie, B. B., Gillette, J. R., and La Du, B. N. Enzymatic metabolism of drugs and other foreign compounds. Annual Review of Biochemistry, 27, 427-454, 1958.

Honors and Awards relating to this project: None

Serial No. NHI-197
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Studies on Short-acting Synthetic Reserpine-like Drugs

Principal Investigators: Dr. Gertrude P. Quinn
Dr. Parkhurst A. Shore

Other Investigator: None

Cooperating Unit: None

Man Years (calendar year 1958):
Total: 5/8
Professional: 5/8
Other: 0

Patient Days (calendar year 1958):
None

Project Description:

Objectives: The tranquilizing effects of reserpine have been associated with the release of serotonin and norepinephrine from brain tissue; an effect attributed by some to the presence of an indole group in the reserpine molecule. A new series of synthetic compounds, quinoli[illegible] derivatives, which have no indole group, have been shown to produce pharmacological effects markedly similar to those of reserpine, including the release of brain amines. The duration of these effects are much shorter than those of reserpine. Further studies with these compounds may increase our knowledge concerning the mechanism of action of reserp[illegible]

Methods Employed: A method has been developed for the estimation of these compounds in biological tissues and fluids.

Major Findings: Most of these studies have been carried out with Tetrabenazine, Ro 1-9596, in rabbits.

In a previous report (-957) it was reported that the duration of tetrabenazine action could be related to the duration of its effects on the brain amines. Also it was shown that tetrabenazine could bl[illegible] some of the pharmacological and biochemical effects of reserpine; thus suggesting that both compounds act on the same effector site

Serial No. NHI-197

The current studies have indicated that tetrabenazine inhibits the reserpine effects by virtue of blocking its action on serotonin and not norepinephrine. Tetrabenazine does not effect these amines in the peripheral tissues, platelets, intestine or heart but does cause some release of catechol amines from the adrenal glands. The latter effect is probably mediated centrally. The peripheral effects of reserpine are not blocked by tetrabenazine.

The short-term biochemical and pharmacological actions of tetrabenazine are related to its half-life in the body which is only 30 minutes. This action may be contrasted to the long-term effects of reserpine which persist even after reserpine can no longer be detected in the body. Therefore, the effects of tetrabenazine are readily reversible whereas those of reserpine are "irreversible".

Significance to the Program of the Institute: This project is an extension and continuation of a long-term program concerning the role of serotonin and norepinephrine in brain function.

Proposed Course of Project: The current project will be continued in an attempt to elucidate further the biochemistry of brain function. Studies of the biological distribution and metabolism of tetrabenazine are planned.

Part B included: No

Serial No. NHI-198
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Studies on the Physiologic and Biochemical Effects of Monoamine Oxidase Inhibitors

Principal Investigators: Dr. Sydney Spector
Dr. Parkhurst A. Shore

Other Investigator: None

Cooperating Units: None

Man Years (calendar year 1958): Patient Days: None
Total: 1-1/8
Professional: 1-1/8
Other: 0

Project Description:

Objectives: Drugs such as iproniazid which inhibit the action of monoamine oxidase are assuming considerable importance in the treatment of depressed mental states, hypertension and angina pectoris. The present work is designed to gain some understanding of the mechanisms by which these drugs exert their actions.

Major Findings: As discussed in a previous report, iproniazid administration causes a marked elevation in the levels of serotonin and a lesser rise in the levels of norepinephrine in the brains of rabbits and rats. The increase in the concentration of these amines, especially norepinephrine, can be related temporally to an excited state observable in these animals. A marked rise in platelet serotonin and a slight rise in heart norepinephrine was also observed. In patients receiving clinical doses of iproniazid, a rise in platelet serotonin has been observed, indicating a blockade of monomaine oxidase in man, even with these small doses.

Serial No. NHI-198

By using a new and very potent amine oxidase inhibitor, phenylisopropylhydrazine, JB 516, a very marked and rapid rise in rabbit brain serotonin occurred, indicating a rapid turnover of this substance in brain (half-life about 10 minutes). Norepinephrine levels rose more slowly, indicating an apparent half-life of about 3 - 4 hours. Central excitation and sympathomimetic responses again occurred when brain serotonin and norepinephrine levels reached 2 - 3 times the normal value.

In cats or dogs, however, no central excitation could be observed after iproniazid or JB 516 treatment. In these species, brain serotonin levels rose rapidly, but little or no change in norepinephrine concentration could be detected. These results would suggest that the elevation of brain norepinephrine levels but not serotonin levels is associated with the central effects.

Significance to the Program of the Institute: These studies are of significance in that they are an attempt to learn more of the mechanism of action of a type of drug demonstrated to be useful in the treatment of various cardiovascular diseases. Furthermore, they may help suggest normal roles for serotonin and norepinephrine, substances implicated in the function of homeostatic mechanisms in the body.

Proposed Course of Project: Further studies will be carried out in an effort to determine which amine, serotonin or norepinephrine, is of major importance in the action of these drugs. Various new types of monoamine oxidase inhibitors will be investigated.

Part B included: Yes

Serial No. NHI-198

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Spector, S., Prockop, D., Shore, P. A., and Brodie, B. B.: The Effect of Iproniazid on Brain Levels of Norepinephrine and Serotonin, Science 127, 704, 1957.

Shore, P. A.: Possible Mechanism of Antidepressant Action of Marsilid, J. Clin. Exp. Psychopath. 19, Supp. 1, p. 56, 1958.

Biel, J., Drukker, A., Shore, P. A., Spector, S., and Brodie, B. B.: Effect of 1-phenyl-2-hydrazinopropane, a Potent Monoamine Oxidase Inhibitor, on Brain Levels of Norepinephrine and Serotonin, J. Am. Chem. Soc., 80, 1519, 1958.

Brodie, B. B., Spector, S., Kuntzman, R., and Shore, P. A.: Rapid Biosynthesis of Brain Serotonin Before and After Reserpine Administration, Naturwissenschaften 45, 243, 1958.

Shore, P. A., Gillespie, L., Spector, S., and Prockop, D.: Increase in Blood Serotonin Levels Induced by Iproniazid in Man and Rabbits, Naturwissenschaften 45, 340, 1958.

Serial No. NHI-199
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: The Metabolism of Amine Uptake and Binding in Blood Platelets

Principal Investigators: Dr. F. Barbara Hughes
Dr. P. A. Shore

Other Investigator: None

Cooperating Units: Dr. Hughes worked under a fellowship -- six months with Geigy Pharmaceuticals and six months with Ciba Pharmaceuticals

Man Years (calendar year 1958): Patient Days: None
Total: 5/8
Professional: 5/8
Other: 0

Project Description:

Objectives: Accumulating evidence points to an essential role for serotonin and norepinephrine in brain function. The amines are said to be "bound" since they are stored in a form which is protected from enzymatic destruction. The question arises as to the nature of the binding and the mechanisms which hold and release these amines. The finding that the same Rauwolfia alkaloids that release serotonin from brain also release it from platelets suggests that the substance is held in both tissues by similar forces. Thus platelets may serve as an in vitro model for studying storage and release of serotonin and catecholamines.

Major Findings: Evidence has been obtained which suggests that serotonin is held within cells by a special mechanism which maintains the amine against a concentration gradient. Reserpine appears to act by interfering with this "pump." By this mechanism, reserpine can release endogenous serotonin from cells or can block the uptake of added serotonin.

Studies with platelets have shown that these cells can also take up epinephrine and norepinephrine, although to a lesser extent than serotonin. The uptake of these catecholamines can be also blocked by reserpine, suggesting that the same mechanism is involved for the release of serotonin and catecholamines by reserpine.

Significance to the Program of the Institute: These findings are relevant to studies of the role of serotonin and norepinephrine in the body.

Proposed Course of Project: The storage and release mechanisms of serotonin and catecholamines in the brain and other tissues are being investigated.

Part B included: Yes

Serial No. NHI-199

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Hughes, F. B., Shore, P. A., and Brodie, B. B.: Serotonin Storage Mechanism and its Interaction with Reserpine. Experientia 14, 178, 1958.

Honors and Awards relating to this project: none

Serial No. NHI-200
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Mechanism of the Excitatory Action of Morphine in Cats

Principal Investigators: Dr. Gertrude P. Quinn
Dr. Parkhurst A. Shore

Other Investigator: None

Cooperating Unit: None

Man Years (calendar year 1958):
Total: 5/8
Professional: 5/8
Other: 0

Patient Days (calendar 1958):
None

Project Description:

Objectives: As a part of a general study of the roles of serotonin and norepinephrine as regulatory agents in the central autonomic nervous system, the effects of morphine, which in cats is excitatory and releases brain norepinephrine, is being compared with reserpine, which causes central depression and releases both serotonin and norepinephrine in brain.

Major Findings: It has been shown that morphine which in cats produces excitement causes the release of norepinephrine but not serotonin from the brain. The results of experiments reported previously would suggest that the excitatory effects of morphine were possibly due to the release of norepinephrine. Further studies, however, have shown that morphine can still produce excitement in reserpinized cats whose brains have been depleted of both norepinephrine and serotonin. Furthermore, the pharmacological and biochemical effects of morphine can be blocked by pretreatment with chlorpromazine. It would appear from the studies with these two tranquilizing agents that morphine is not dependent upon norepinephrine for its effects and that the release of norepinephrine is the result of the excitation.

Serial No. NHI-200

Significance to the Program of the Institute: This project is a part of a larger program investigating the actions of various centrally acting drugs and the possible function of serotonin and norepinephrine.

Proposed Course of Project: The studies directed toward the elucidation of the mechanism of action of morphine will be continued. Other excitatory agents will be used and their influence on norepinephrine and serotonin will be investigated. Also other biological systems, such as cholinesterase and compounds influencing these systems will be studied.

PART B included: No

Serial No. NHI-201
1 Chemical Pharmacology
2 Biochemistry of Drug Action
3 Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Studies on the Biochemistry and Physiology of Histamine

Principal Investigators: Dr. P.A. Shore
Dr A. Burkhalter
Mr. V.H. Cohn

Other Investigator: None

Cooperating Unit: None

Man Years (calendar year 1958):
Total: 1-5/8
Professional: 1-1/8
Other: 1/2

Patient Days (calendar year 1958):
None

Project Description:

Objectives: It has been known for many years that histamine occurs in the body and that it is a remarkably potent substance, causing such diverse effects as hypotension, changes in capillary permeability, increased gastric acid secretion and stimulation of certain parts of the central nervous system. In spite of the large amount of data collected concerning this substance, little is known definitely of some fundamental biochemical factors, or of possible functions in the body. One reason for the relative paucity of finding in certain areas is that sensitive and specific methods for its estimation have not been available. We have therefore turned our attention to a search for a sensitive analytical technique. Once this has been accomplished, investigations into the biochemistry and physiology of histamine will be launched.

Major Findings: An extremely sensitive (0.01 γ/cc) fluorometric technique for the estimation of histamine has been discovered. The sensitivity compraes well with the very sensitive, but laborious and sometimes non-specific bioassay procedures. The technique appears to be specific for histamine even in the presence of substances such as serotonin and catecholamines.

Serial No. NHI-201

Preliminary results indicate that previously developed chemical techniques for the estimation of tissue histamine may be non-specific and include substances other than histamine.

Significance to the Program of the Institute: Because of the very marked effects of histamine on the cardiovascular system, there is a need for more knowledge of the factors involved in its metabolism and actions.

Proposed Course of Project: After perfection of the fluorometric method as applied to tissues, it is planned to launch an investigation into the biosynthesis and metabolism of histamine and the effect of various drugs on its metabolism and physiological actions. It is expected that this project will be expanded in the near future.

Part B included: No

Serial No. NHI-202
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Biochemical Mechanisms of Action of Reserpine and Related Compounds

Principal Investigators: Dr. K.F. Finger
Dr. F.B. Hughes

Other Investigator: None

Cooperating Units: Chas. Pfizer and Co., salary for Dr. Finger; Ciba Pharmaceutical Laboratories, salary for Dr. Hughes

Man Years (calendar year 1958):
Total: 1-1/2
Professional: 1-1/2
Other: 0

Patient Days (calendar year 1958): None

Project Description:

Objectives: Interpretation of peripheral and central effects of Rauwolfia alkaloids in terms of neurohumoral agents.

Methods Employed: Assay of norepinephrine and serotonin; routine pharmacological procedures.

Major Findings: Our findings have made it necessary to modify the general opinion that the sedative and hypotensive actions of reserpine have a common central mechanism. Reserpine exerts two distinct and separate actions, one peripheral and the other central.

Thus reserpine administered intravenously to rabbits in doses of 10 γ/kg depletes heart norepinephrine without having much effect on brain norepinephrine and serotonin. These low doses of drug lower sympathetic activity without producing sedation.

Serial No. NHI-202

A more complete separation of peripheral and central effects was found with SU 3118 (Ciba). This carbethoxy syringoyl substitutes methyl reserpate in daily doses of 50 γ/kg, depletes heart norepinephrine in rabbits and dogs without affecting levels of brain amines. Animals are not sedated, but exhibit hypotension, bradycardia and a markedly diminished response to occlusion of the carotid sinus. Furthermore, it reduces the sympathetic responses to adrenergic stimulation and to ganglionic stimulation.

It is concluded from these results reserpine-like compounds do not lower sympathetic by central action, but by peripheral depletion of norepinephrine which render sympathetic organs incapable of responding to stimuli.

Preliminary studies with reserpine indicate when the drug is given orally only a small percentage enters the bloodstream. It is possible that the difficulty in adjusting dosage in hypertension so that patients are not also depressed, is due to the variability in the degres of metabolism in the gut. Attempts arebeing made, in collaboration with Ciba, to make a more stable reserpine analog, perhaps not an ester. Ideally such a compound will be completely absorbed and will depress blood pressure in doses that do not elicit sedation.

Norepinephrine depletion does not account for sedation and other central effects of reserpine. Thus, SU 5171 (Ciba), a demethylamino benzoylmethyl reserpate, almost completely depletes brain norepinephrine in rabbits when given in doses of 0.5 mg per kg, but has little effect of brain serotonin. The anim ls are not sedated, but 2 mg of drug per kg causes a 65% decrease in brain serotonin and a definite sedation is evident. Recovery from sedation coincides with rise in serotonin levels despite continued low brain norepinephrine. Thus SU 5171 is a valuable tool in showing that changes in brain serotonin rather than on norepinephrine may be the important factor in the central effects of reserpine. This data supports the thesis that reserpine affects a neuronal system for which serotonin is the hormone; in contrast to chlorpromazine which affects a central adrenergic system.

Significance to the Program of the Institute: The study of these and other analogs will contribute to the understanding of the hormones involved in central homeostatic mechanisms and may lead to the development of more specific and efficacious therapeutic agents for the control of cardiovascular diseases.

Proposed Course of Project: To continue thecorrelation of pharmacological responses with the observed biochemical changes occurring in the brain and the heart, and in addition, to follow the metabolism of these analogs by both in vivo and in vitro methods in an attempt to correlate changes in the chemical structure with observed responses. In addition, apply findings to synthesis of compounds which have only peripheral or only central actions and whose effects are more predictable in clinical hypertension or in mental disease.

Part B included: No

Serial No. NHI-203
1. Chemical Pharmacology
2. Biochemistry of Drug Action

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Studies on the Biosynthesis of Catecholamines in the Brain

Principal Investigators: Dr. P.A. Shore
Dr. D.F. Bogdanski
Mr. R. Kuntzman

Other Investigator: None

Cooperating Unit: None

Man Years (calendar year 1958):
Total 7/8
Professional: 3/8
Other: 1/2

Patient Days (calendar year 1958):
None

Project Description:

Objectives: The question as to the function of catecholamines in the brain has been assuming an increasing importance. There is reason to believe that functional excesses or deficits of these substances in brain may be reflected in a variety of syndromes and responses to drugs

Major Findings: Studies on the distribution of norepinephrine have been described in the report entitled "Studies on the Identification and Distribution of Catecholamines in the Body". Studies have also been carried out on the distribution, within the brain, of the enzyme dopa (3,4-dihydroxyphenylalanine) decarboxylase, one of the enzymes involved in the formation of catechclamines. It has been found that the distribution of the enzyme activity parallels roughly the norepinephrine concentration of the various portions of the brain. An exception is the basal ganglion, which shows an extremely high enzyme activity but a rather low norepinephrine level. This is of interest in view of the recent discovery in Sweden that dopamine (3,4-dihydroxyphenylethylamine) occurs in very high concentration in portions of the basal ganglion.

The distribution of dopa decarboxylase activity also parallels the activity of 5-hydroxytryptophan decarboxylase. This observation

is consistent with the possibility that these enzymes are identical.

Studies have been carried out with compounds, such as thiosalicylic acid, which inhibit in vitro the action of dopa decarboxylase. It has been found that these substances also inhibit the decarboxylation of 5-hydroxytryptophan.

Significance to the Program of the Institute: These studies are a part of a general inquiry into the biochemistry and function of catecholamines in brain.

Proposed Course of Project: Attempts will be made to inhibit in vivo the activity of brain dopa decarboxylase. If this should prove possible, it is planned to deplete the stores of catecholamines from the brain. From the ensuing pharmacologic effects, an understanding of the role and importance of catecholamines in the brain and in the action of reserpine might be forthcoming.

Part B included: No

Serial No. NHI-204
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Relationship between Monoamine Oxidase Inhibition, Barbiturate Action and Local Anesthesia

Principal Investigator: Dr. Marie-Jeanne Laroche

Other Investigator: None

Cooperating Unit: Dr. Laroche's salary paid from NIH Fellowship

Man Years (calendar year 1958):
Total: 1
Professional: 1
Other: 0

Patient Days (calendar year 1958): None

Project Description:

Objectives: This laboratory has previously shown that Marsilid, a monoamine oxidase inhibitor, prolongs the duration of barbiturate action in mice. Since inhibitors of monoamine oxidase all have this property, the question arises as to whether it is related to the blocking of barbiturates.

Local anesthetics are characterized by their high lipid solubility and by their ability to inhibit monoamine oxidase. It is possible that blocking of this enzyme in certain nerves may be associated with blocking of nerve conduction which may be mediated by an amine neurohormone.

Methods Employed: Methods for measuring monoamine oxidase activity and for assaying local anesthetic activity.

Major Findings: A number of structurally different monoamine oxidase inhibitors have been found to increase the duration of action

Serial No. NHI-204

of hexobarbital. However, this effect is not due to blocking of monoamine oxidase, since it is maximal immediately following administration of inhibitor at a time when monoamine oxidase is barley depressed, and has disappeared when the inhibition of monoamine oxidase, is complete. This action of monoamine oxidase appears to be a reversible one, presumably a result of inhibiting the metabolizing enzyme in the microsomes.

Preliminary investigations have shown that a number of local anesthetics, e.g., cocaine, procaine and xylocaine, are monoamine oxidase inhibitors. Certain compounds that are clinically used as anti-depressant monoamine oxidase inhibitors, e.g., JB 516 and Marsilid when administered topically increased the anesthetic activity of cocaine and in fact when administered under the skin of a guinea pig exhibited some local anesthetic effect.

Significance to the Program of the Institute: These findings relevant to the role of monoamine oxidase, norepinephrine and serotonin in the mechanism of action of local anesthetics.

Proposed Course of Project: To continue studies on the mechanism of action of local anesthetics.

Part B included: No

Serial No. NHI-205
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Studies on Anti-convulsant Activity of Monoamine Oxidase Inhibitors

Principal Investigators: Dr. Darwin J. Prockop
Dr. P.A. Shore

Other Investigator: None

Cooperating Unit: Life Insurance Medical Research Fund
(supplied fellowship for Dr. Prockop)

Man Years (calendar year 1958):
Total: 7/8
Professional: 7/8
Other: 0

Patient Days (calendar year 1958):
None

Project Description:

Objectives: Reserpine, which lowers the concentration of serotonin and norepinephrine in the brain, has been reported to lower the convulsive threshold to such convulsant stimuli as electroshock and Metrazol. The temporal correlation between onset and duration of action of this facilitating effect and the depletion of brain amines is quite striking.

Since it has been observed that monoamine oxidase inhibitors increase the concentration of these amines in the brain (see report) entitled "Studies on the Physiologic and Biochemical Effects of Monoamine Oxidase Inhibitors"), it was felt that there was a possibility that these inhibitors might act as anti-convulsant agents.

Major Findings: It has been found that various inhibitors of monoamine oxidase protect against maximal electroshock seizures in rats. Three inhibitors were tested: iproniazid, JB 516 (phenylisopropylhydrazine) and JB 807 (1-phenyl-2-(isopropylhydrazine)propane. A close correlation was found between the anticonvulsant activity

and the increase in brain levels of serotonin and norepinephrine; however, neither the serotonin precursor, 5-hydroxytryptophan, nor dihydroxyphenylalanine, a precursor of norepinephrine, affected the response to electroshock. Pretreatment of rats with the inhibitors reversed the facilitating action of reserpine. The inhibitors also blocked Metrazol-induced seizures in mice, but not strychnine-induced seizures, indicating that the site of the anti-convulsant action is central.

Significance to the Program of the Institute: This project is designed to further the understanding of possible roles of serotonin and nore pinephrine and possible mechanisms involved in the action of reserpine and monoamine oxidase inhibitors.

Part B included: No

Serial No. NHI-206
1. Chemical Pharmacology
2. Cell Permeability
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Absorption of Natural Products from the Gastrointestinal Tract

Principal Investigator: Dr. Lewis S. Schanker

Other Investigator: Mr. Dominick J. Tocco

Cooperating Units: None

Man Years (calendar year 1958): Patient Days: None
Total: 1-1/4
Professional: 1/4
Other: 1

Project Description:

Objectives: To broaden our understanding of how a number of water-soluble substrates, especially the nucleic acid derivatives, cross biological membranes.

Methods Employed: Solutions of various nucleic acid derivatives in isotonic saline (pH 7.2) were continuously recirculated through the entire small intestine of the anesthetized rat. The degree of absorption was determined by the decrease in concentration after 1 or 2 hours of recirculation.

Major Findings: Previous work on this project indicated that specialized transport mechanisms might be involved in the intestinal absorption of several purine and pyrimidine bases and nucleosides. These preliminary results, however, were complicated by the observation that some of the compounds under investigation were not completely stabile in the intestinal solutions.

In the work now reported, only those compounds that were shown to be stabile when incubated with intestinal contents were studied in detail.

Serial No. NHI-206

The intestinal absorption of the pyrimidine base thymine from solutions of various concentrations indicates that a transport mechanism is involved. Thus the degree of absorption at low concentrations of thymine deviates from Fick's law of passive diffusion, and the absorption process becomes saturated at higher concentrations. Similar kinetics were observed for the nucleoside thymidine. The absorption of thymine and thymidine follows the Michaelis-Menten kinetics and yields straight lines when subjected to the Lineweaver-Burke plot.

When thymine (5-methyluracil) absorption was followed in the presence of varying concentrations of uracil, there was a marked depression of thymine absorption. A Lineweaver-Burke plot of the results indicates that the inhibition is competitive in nature. Preliminary results suggest that a number of purine and pyrimidine derivatives may also be competing for the same transport mechanism. High concentrations of D-glucose or L-histidine do not appear to inhibit the transport of thymine, suggesting that the transport mechanism for thymine is different from those responsible for the active intestinal absorption of sugars and L-amino acids.

Significance to the Program of the Institute: Knowledge of the means by which natural substrates required by the cell traverse cell membranes should broaden our understanding of the functions of the cell and its components.

Proposed course of Project: To further examine the transport of nucleic acid derivatives and related synthetic compounds across the intestinal epithelium as well as other body membranes.

Part B included: No

Serial No. NHI-207
1. Chemical Pharmacology
2. Cell Permeability
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Absorption of Drugs by the Small Intestine

Principal Investigator: Dr. Lewis S. Schanker

Investigator (Other): Mr. Panayotis A. Nafpliotis

Cooperating Unit: None

Man Years (calendar year 1958):
Total: 3/4
Professional: 1/4
Other: 1/2

Patient Days (calendar year 1958):
None

Project Description:

Objectives: To determine the factors governing the intestinal absorption of drugs.

Methods: Solutions of drugs in isotonic saline solution were perfused through the small intestine of the anesthetized rat. The degree of absorption of a drug was determined by measuring the decrease in drug concentration after a single passage through the intestine.

Major Findings: Previous work on this project demonstrated that drugs are most rapidly absorbed from the small intestine if they are present in their unionized form, whereas the ionized form is absorbed with difficulty.

These results also suggested that some factor other than the degree of ionization of a drug is also important since various unionized compounds are absorbed at different rates. Classical

Serial No. NHI-207

experiments on the permeability of cell membranes have suggested that membranes in general are lipoidal in nature since fat-soluble substances penetrated the cell more readily than fat-insoluble substances.

The present study has dealt with the relation between the lipid-solubility of drugs and their rates of absorption. To eliminate the variable of the degree of ionization of various drugs, a large group of weak organic electrolytes which would be unionized in the intestine were selected for the study. The relative rates of absorption were compared with the lipid:water partition coefficients of the unionized drugs using chloroform or heptane as the "lipid" phase.

The results indicate a rough relation between lipid-solubility and the degree of absorption of a drug. The compounds that were rapidly absorbed had relatively high lipid-solubilities while slowly absorbed drugs had very low lipid-solubilities.

These results suggest that the intestinal mucosa is lipoid in character. Thus for the most rapid absorption, a drug should be largely unionized in the intestinal lumen, and the unionized drug should be highly fat-soluble.

Significance to the Program of the Institute: These studies should serve as useful guidelines for the synthesis of new drugs which will be highly effective when administered by the oral route.

Proposed Course of Project: (1) Investigate the absorption of quaternary ammonium compounds. (2) Investigate the possible role of chelation in the absorption of certain drugs. (3) Investigate the absorption of certain antibiotic drugs which are only moderately well absorbed in therapeutics.

Part B included: Yes

Serial No. NHI-207

Publications:

Schanker, L.S., Tocco, D.J., Brodie, B.B., and Hogben, C.A.M., Absorption of drugs from the rat small intestine. J. Pharmacol. and Exp. Therap., 123, 81, 1958.

Honors and Awards: None

Serial No. NHI-208
1. Chemical Pharmacology
2. Cell Permeability
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Absorption of Drugs from the Colon

Principal Investigator: Dr. Lewis S. Schanker

Other Investigator: Mr. Panayotis A. Nafpliotis

Cooperating Units: None

Man Years (calendar year 1958): Patient Days: None
Total: 3/4
Professional: 1/4
Other: 1/2

Project Description:

Objectives: To describe the characteristics of the colonic mucosa which govern its permeability to drugs and other substances.

Methods Employed: The colon of the anesthetized rat was cannulated at the cecal and rectal ends, and perfused with solutions of various drugs. The degree of absorption of a drug was determined by measuring the decrease in drug concentration after a single passage through the colon.

Major Findings: Earlier work on this project indicated that the degree of absorption of organic electrolytes from the rat colon is related to the dissociation constant of the compound. Thus weak acids and bases were, in general, readily absorbed while stronger acids and bases were very poorly absorbed. These observations suggested that the colonic mucosa is preferentially permeable to the unionized form of a drug and that ions penetrate with difficulty. The present report describes studies which support this working hypothesis and which suggest that the colonic mucosa is lipoid in character.

The absorption of acidic drugs such as salicylic and benzoic acids was greatly enhanced when they were perfused through the colon in an acidic solution (pH 4). In contrast, the absorption of basic drugs like aniline or quinine was decreased at pH 4. Thus a change in the intralumenal pH which increases the proportion of unionized drug facilitates absorption; conversely, decreasing the proportion of unionized drugdiminishes the degree of absorption.

Serial No. NHI-208

Further support for the conception of a membrane selectively permeable to the unionized form of a drug was obtained by determining the steady state distribution of quinine in plasma and colonic lumen. The observed concentration ratio (gut:plasma) of 7.8 is very close to the value predicted for such a membrane when it separates solutions of the measured pH values of plasma and gut solution.

The partitions of a large number of drugs between a lipid solvent (chloroform or heptane) and water were measured under conditions where the drugs would be completely unionized. There is a sufficient parallel between lipid-solubility of drugs and their degree of absorption to suggest that the mucosa of the colon is lipoid in character.

The conception of a lipoid barrier is consistent with the observation that the lipid-soluble unionized form of a drug is readily absorbed while the lipid-insoluble ionized form is absorbed with difficulty.

When drug solutions of widely different concentrations were perfused through the colon, the amount of drug absorbed was directly proportional to the concentration. This suggests that absorption is occurring by simple diffusion.

Thus absorption of drugs from the rat colon can be explained as the passive diffusion of unionized drug moieties across a lipoidal membrane.

Significance to the Program of the Institute: The characterization of cell membranes regarding their permeability to drugs and other substances allows us to select or synthesize therapeutic agents which will most readily reach their site of action in a sufficient concentration to exert the desired pharmacologic effect.

Proposed Course of Project: Examine the absorption of sugars and amino acids from the colon.

Part B included: No

Serial No. NHI-209
1. Chemical Pharmacology
2. Cell Permeability
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: The Secretion of Substances into Bile

Principal Investigator: Dr. Lewis S. Schanker

Other Investigator: None

Cooperating Unit: In collaboration with Dr. C. Adrian M. Hogben, Dept. of Physiology, The George Washington University School of Medicine, Washington, D.C.

Man Years (calendar year 1958):
Total: 1/8
Professional: 1/8
Other: 0

Patient Days (calendar year 1958): None

Project Description:

Objectives: To describe the means by which substances pass from the bloodstream into the bile.

Methods: The bile duct of the anesthetized rat was cannulated and the renal pedicles were ligated. After an intravenous injection of a C^{14} labeled saccharide, bile was collected for 3 to 5 hours. The radioactivity in bile samples was compared with that in plasma.

Major Findings: Previous work on this project disclosed that 2 large lipid-insoluble saccharides, inulin and sucrose, are secreted into bile in significant concentrations. This suggested a significant porosity at some locus in the hepatobiliary system.

In the work now reported, the biliary secretion of inulin, sucrose and a smaller molecule, mannitol, were examined in more detail and the distribution of these substances in liver and muscle tissue was also determined. The results are summarized in the following table.

Serial No. NHI-209

Substance	Bile:Plasma Concentration Ratio	Liver Space (% of wet weight)	Muscle Space (% of wet weight)
Inulin	0.09	24%	12%
Sucrose	0.21	23%	11%
Mannitol	1.16	72%	13%

The results suggest the presence of relatively large pores in the "blood-bile barrier", the very large inulin molecule penetrating the barrier significantly but relatively slowly and smaller molecules penetrating more readily. Mannitol, the smallest molecule studied, appears to be distributed in the total water of the liver; inulin and sucrose appear to occupy a liver space greater than the extracellular space determined by other investigators.

Significance to the Program of the Institute: These studies may lead to an understanding of the biliary secretion of drugs and metabolites.

Proposed Course of Project: (1) Investigate further the permeability of hepatic cells to lipid-insoluble molecules like inulin and sucrose. (2) Study the biliary secretion of drugs and metabolites.

Part B included: No

Serial No. NHI-210
1. Chemical Pharmacology
2. Cell Permeability
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A

Project Title: Penetration of Drugs into the Pituitary and Pineal Glands

Principal Investigator: Dr. Cedric W.M. Wilson
Dr. Lewis S. Schanker

Other Investigator: None

Cooperating Unit: Dr. Wilson's fellowship is from the Medical Research Council of Great Britain and is sponsored from Eli Lilly.

Man Years (calendar year 1958):
Total: 1/4
Professional: 1/4
Other: 0

Patient Days (calendar year 1958):
None

Project Description:

Objectives: Relationship of the pineal and pituitary glands to the rest of the brain.

Methods Employed: Drugs are administered intravenously to cats The animals are sacrificed at various intervals and portions of the brain removed for measurement of drug content.

Major Findings: When sulfaguanidine was given intravenously to the cat, it passed into the pituitary and pineal glands and perhaps into the area postrema as readily as into peripheral tissues, but penetrated other areas of the brain, such as the cerebral cortex and medulla, with difficulty.

Earlier studies by this laboratory demonstrated that the central nervous system is separated from the bloodstream by a lipoid-like barrier. Therefore the finding that the pituitary and pineal bodies are relatively easily penetrated by a lipid-insoluble, foreign substance suggests that the "blood-brain barrier" may be absent in these areas which are generally considered to be part of the central nervous system. This suggests that these areas are not parts of the brain. Since

the pituitary is a secretory organ, it is possible that the pineal body and the area postrema also possess this function.

Significance to the Program of the Institute: The finding of brain structures which appear to have permeability characteristics quite different from the central nervous system as a whole is highly important in view that little is known of the control of pituitary function.

Proposed Course of Project: (1) Develop suitable microanalytical techniques for determining the distribution of drugs and other substances in certain small areas of the brain such as the pineal and pituitary bodies, and the area postrema.

(2) Study the permeability characteristics of these brain structures.

(3) Investigate the possibility that the pineal glad and the area postrema may be secretory structures.

Part B included: No

Serial No. NHI-211
1. Heart
2. Chemical Pharmacology
3. Clinical Pharmacology
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Intravenous Anesthetics.

Principal Investigator: Dr. Peter G. Dayton

Other Investigators: Dr. J.J. Burns
Mrs. Dolores Taller

Cooperating Units: In collaboration with the Department of Anesthesiology, College of Physicians and Surgeons, Columbia University, and New York University, Research Service, Goldwater Memorial Hospital, New York, New York.

Man Years (calendar year 1958):
Total: .28
Professional: .28
Other: None

Patient Days (calendar year 1958): None.

Project Description:

Objectives - A study of the physiological disposition and intermediary metabolism of various barbiturates is intended to derive fundamental information concerning the pharmacology of intravenous anesthetics. There is a need for a potent intravenous anesthetic which may be used in surgical procedures of long duration. In this respect, an effort is being made to find a non-barbiturate anesthetic, since it has become clear that barbiturates as a class are slowly metabolized and exert a hypnotic and not a truly anesthetic action. In addition, barbiturates have other drawbacks (laryngospasm, depressed respiration, poor muscular relaxation, ganglionic blockade, etc.)

Patient Material - None.

Major Findings - The site and mechanism of formation of the cerebrospinal fluid and the manner in which foreign substances enter and leave it are controversial matters. The barbiturates enter and leave the brain at different rates in proportion to their degree of lipid solubility. It seemed reasonable to look for differences also in their rates of passage into and out of the cerebrospinal fluid. So far only barbital has been studied extensively in this direction. (Barbital was chosen because it distributes uniformly in total body water). Experiments currently in progress in dogs suggest that barbital passes slowly from the bloodstream into the brain and thence into the cere-

brospinal fluid as an extension of the extra cellular water of the brain. This conclusion, if verified, would seem to negate the importance of the choroid plexus as an active mechanism for transfer of this foreign substance at least from blood to cerebrospinal fluid.

Significance to the Program of the Institute - Information on how intravenous anesthetics, such as thiopental, distribute in body is of importance in understanding their action in clinical anesthesiology.

Proposed Course of Project - Studies are being carried out on the effect of changes in pH of the blood on the physiological disposition of thiopental. We have previously demonstrated in dogs a decline in plasma levels of thiopental with decline in pH induced by carbon dioxide inhalation. Since the depressant effects of thiopental on the brain are the basis for its clinical use, it seemed important to examine the changes in drug concentrations in the brain during the same alterations in pH of the blood. This study, just begun, should have both theoretical value and practical usefulness, for ventilatory errors during barbiturate anesthesia may well be of sufficient magnitude to produce corresponding changes in man.

Part B included Yes

(Attachment 1)

Serial No. NHI-211

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Mark, L.C., Burns, J.J., Brand, L., Campomanes, C.I., Trousof, N., Papper, E.M. and Brodie, B.B.: The passage of thiobarbiturates and their oxygen analogs into brain. J. Pharm. and Exptl. Thera., 123: 70-73, 1958.

Honors and Awards relating to this project: None.

Serial No. NHI-212
1. Heart
2. Chemical Pharmacology
3. Organic Chemistry
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: The Metabolism of 6-Chloropurine (6-ClP).

Principal Investigators: Dr. Daniel Duggan
Dr. Elwood O. Titus

Other Investigator: None.

Cooperating Units: None.

Man Years (calendar year 1958):
Total: 1.25
Professional: 1.25
Other: None

Patient Days (calendar year 1958): None.

Project Description:

Objectives - The immediate objective of this project is to find, through a preliminary study of the in vitro and in vivo metabolism of 6-ClP, the most promising approach to the problem of how this drug and similar purine antimetabolites are involved in the enzymic control of cellular processes.

Methods Employed - To date, assays of starting materials and metabolites have been accomplished by conventional spectrophotometric, manometric, chromatographic and radioisotopic techniques, obviating the necessity of developing new, specific assay procedures.

Patient Material - None.

Major Findings -

A. In Vitro

6-ClP has been found to be a competitive inhibitor of purine oxidation by liver - and milk xanthine oxidase (XO). The milk XO slowly oxidized 6-ClP to 6-chlorouric acid (6-ClU), a heretofore unknown compound, which has been obtained in crystalline form and characterized by indirect chemical evidence (conversion by unambiguous route to known compound, study of acid dissociation constants) in addition to ultraviolet and infra-red spectrophotometric evidence. 6-ClU was found to be an extremely potent inhibitor of uricase. Appropriate binding and inhibition constants for 6-ClP and and 6-ClU have been calculated.

6-ClP, unlike 6-mercaptopurine does not catalyze the enzymic oxidation of inorganic sulfite by milk XO, and, although an inhibitor of hypoxanthine oxidation by XO, is completely without effect upon the hypoxanthine - catalyzed "sulfite oxidase" system. This is a unique feature of 6-ClP as compared with other known XO inhibitors.

B. In Vivo

6-ClP-8-C^{14} was prepared from hypoxanthine-8-C^{14} by known procedures. Using tracer dosages in adult rats, the following results have been obtained to date:

(a) In recovery experiments, over 90% of total isotope was accounted for in urine, expired CO_2 and tissues.

(b) Chromatography of urine reveals the presence of four major excretory products, three of which have been identified as starting drug, 6-ClU and uric acid. These three products have been estimated quantitatively by precipitation with unlabeled carrier, and correspond respectively to 62, 21 and 6.5% of total urinary excretion of isotope.

(c) Tissue distribution of total isotope has revealed no marked asymetric distribution when differences in mitotic rate are taken into account.

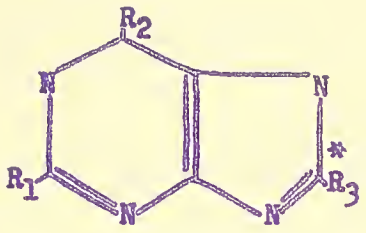
(d) Preliminary experiments have indicated a small extent of incorporation of isotope into purified nucleic acid fractions.

Significance to the Program of the Institute - This project extends the studies of drug metabolism, which have been under way for some years in this laboratory, to compounds which are structurally very similar to normal metabolites. It is hoped that systematic study of the metabolism of such compounds by so called "normal" enzymes will aid in identifying enzymatic processes which are involved in the action of the drugs and in the development of drug resistance.

Proposed Course of Project - To refine techniques employed in in vivo studies above to identify all metabolites of 6-ClP under conditions where both tracer and therapeutic dosages are adminstered and find differentiation, if any, in the mode of metabolism in various tissues. Investigation of the possible replacement by 6-ClP as such, or by one of its unnatural metabolites of the normal purine constituents of nucleic acids and co-factors will be made, and the effects of these abnormal substances upon cellular processes studied.

Part B included No

Serial No. NHI-212



* C-8 position

	R_1	R_2	R_3
Hypoxanthine	H	OH	H
Uric acid	OH	OH	OH (keto)
6-Chloropurine	H	Cl	H
6-Chlorouric acid	OH	Cl	OH
6-Mercaptonurine	H	SH	H (keto)

Serial No NHI-213
1. Heart
2. Chemical Pharmacology
3. Clinical Pharmacology
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A:

Project Title: Studies on Induced Enzyme Synthesis in Mammals.

Principal Investigator: Dr. Allan Conney

Other Investigators: None.

Cooperating Unit: None.

Man Years (calendar year 1958):
Total: .25
Professional: .25
Other: None

Patient Days (calendar year 1958): None.

Project Description:

Objectives - (a) To study the effect of polycyclic hydrocarbons on the induced synthesis of various liver microsomal enzymes which metabolize drugs.

(b) To determine whether various drugs can induce the synthesis of microsomal enzymes which metabolize foreign compounds.

Patient Material - None.

Major Findings - Previous work has shown that the administration of certain polycyclic hydrocarbons (3-methylcholanthrene, 3,4-benzpyrene, and 1,2,5,6-dibenzanthracene) to rats will markedly increase the activity of liver microsomes to, 1) hydroxylate 3,4-benzpyrene, 2) N-demethylate aminoazo dyes, and 3) to reduce the azo linkage of aminoazo dyes. These enzyme systems are similar to many drug metabolizing enzymes, for they are localized in liver microsomes and require TPNH and oxygen. The possibility was investigated that 3,4-benzpyrene may also increase the activity of microsomal enzyme systems which metabolize drugs. It was found that this hydrocarbon markedly increased the activity of the microsomal enzyme system which hydroxylates acetanilide and Flexin. However, 3,4-benzpyrene has a lesser effect on the enzymes which hydroxylate quinoline, naphthalene, and paraflex. Little or no effect was observed on the enzyme systems which demethylate N-methyl aniline and monomethyl-4-aminoantipyrine or which oxidize p-nitrotoluene and chlorpromazine. These studies point out the specificity of 3,4-benzpyrene on the induced synthesis of microsomal enzymes and suggest the presence of families of enzymes carrying out similar reactions. For example, with 3,4-benzpyrene administration hydroxylation of several substrates are affected to different extents.

Further studies on the effect of drugs on microsomal enzyme systems have shown that the administration of Chloretone or barbital markedly increases the activity of the rat liver enzyme which N-demethylates aminoazo dye but has little or no effect on the enzyme which reduces the azo linkage of aminoazo dyes. The possibility that polycyclic hydrocarbons and drugs stimulate ascorbic acid biosynthesis by inducing enzyme synthesis has been discussed in the project report on the Glucuronic Acid Pathway of Glucose Metabolism.

Significance to the Program of the Institute - Studies on induced enzyme synthesis will give information on the general problem of how foreign compounds are metabolized in the body.

Proposed Course of Project - (a) To further study the effect of hydrocarbons on the induction of synthesis of enzymes which metabolize drugs.

(b) To study the effects of various foreign compounds other than hydrocarbons on the induced synthesis of various microsomal enzymes. The interesting possibility, that a drug may be capable of inducing the synthesis of enzymes involved in its own metabolism, will be investigated.

Part B included No

Serial No. NHI-214
1. Heart
2. Chemical Pharmacology
3. Clinical Pharmacology
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A.

Project Title: Studies on Antirheumatic Drugs.

Principal Investigator: Dr. J.J. Burns

Other Investigators: Dr. Peter G. Dayton
Dr. L. Sicam
Miss Dolores Taller
Mr. Miguel Landrau

Cooperating Units: In collaboration with New York University Research Service at Goldwater Memorial Hospital, Department of Medicine, Mount Sinai Hospital, New York and Geigy Laboratories, Basel, Switzerland.

Man Years (calendar year 1958):
Total: .28
Professional: .28
Other: None.

Patient Days (calendar year 1958):
None.

Project Description:

Objectives - Phenylbutazone, a synthetic pyrazolone derivative, has found considerable use in the treatment of various arthritic diseases. We reported previously that this drug has antirheumatic effects comparable to that of cortisone. Phenylbutazone produces marked retention of sodium, but its action is apparently not mediated through the pituitary-adrenal axis, since it does not affect eosinophiles, urinary 17-ketosteroids, potassium excretion, or produce signs of hypoadrenalism. Although phenylbutazone is a very potent antirheumatic agent, its usefulness is limited by such side effects as edema, gastrointestinal hemorrhage, skin reactions and occasionally agranulocytosis. A simple non-steroidal molecule with the antirheumatic effects of phenylbutazone, but lacking its undesirable side effects, would be of paramount importance to the therapy of rheumatoid arthritis, rheumatic fever, gout and related musculoskeletal disorders. A collaborative search for such a drug has been undertaken with Geigy Pharmaceuticals. Promising compounds which have been screened for anti-inflammatory effect in animals will be tested in patients with active arthritis.

Patient Material - None.

Major Findings - Various compounds have been synthesized and tested for anti-imflammatory activity and acute toxicity in animals by the Geigy Laboratory in Basel, Switzerland. These compounds are first studied at Goldwater Memorial Hospital to see whether they have a suitable physiological disposition and to obtain information for establishing a rational regime of therapy for clinical trials to evaluate anti-inflammatory activity in rheumatoid arthritis. The effect of these compounds in acute and chronic gout is studied by Dr. Alexander B. Gutman and Dr. Tsai Fan Yü at Mount Sinai Hospital, New York City. When compounds look promising, chronic toxicity studies are made and extensive clinical trials are conducted by a number of collaborating groups.

Up to the present, 61 analogues of phenylbutazone have been studied in respect to their physiological disposition, their anti-rheumatic properties in acute gout and rheumatoid arthritis and their effect on urinary excretion of sodium and uric acid. Results obtained so far gives us an idea of what structural features are required in the molecule for the various pharmacological actions of phenylbutazone.

(a) Modifications in phenylbutazone, resulting in increased acidity of the molecule are associated with enhanced uricosuric activity, whereas changes which decrease the acidity of the drug are accompanied by loss of this activity. This structural activity relationship has been confirmed during the past year by experiments with two key compounds G-13838, an isopropyl analogue which is the weakest acid in the series (pK 5.5), had no uricosuric activity. On the other hand, G 32567, a p-dimethyl sulfone derivative (pK 2.8) has potent uricosuric activity.

The potent uricosuric activity of the highly acidic phenylbutazone analogue indicates that these drugs act in the ionic form to block the reabsorption of uric acid by the renal tubule cells, thus furnishing a possible clue to the still obscure nature of the transport mechanisms involved. The demonstration that uricosuric activity in the phenylbutazone series is related to the pKa, has proved of considerable aid in searching for new uricosuric drugs. One such drug, a sulfoxide (G-28315), has already found to be useful clinically as a potent uricosuric agent for the treatment of gout and it will be introduced shortly for this purpose as Anturan.

(b) Previously we reported that Metabolite I formed by introduction of a hydroxyl group into the para-position of a benzene ring of phenylbutazone has potent antirheumatic and sodium retaining activity. This compound has undergone extensive clinical trials in various arthritic clinics and appears now to be a more potent and a less toxic antirheumatic agent than phenylbutazone. This compound will be introduced shortly as a new drug for the arthritic diseases.

Further studies have shown that substitutions of other groups into the para position i.e. chloro, methyl or nitro, also result in drugs having the potent antirheumatic and sodium retaining effects of phenylbutazone. A possible exception appeared to be G-32567, a p-dimethyl sulfone derivative. However, this compound disappeared from the body so rapidly (half-life 1 hour) that effective plasma levels could not be maintained even with frequent and large doses. Substitution of hydroxyl or chloro groups into the meta position of the benzene ring yielded drugs having little or no antirheumatic or sodium retaining effects. These observations point out the marked difference that meta and para substitutions in the molecule have on the pharmacological properties of the drug. This point was further tested with G-32961, which has a hydroxyl group in the meta position of one benzene ring and a methyl group in the para position of the other. This analogue was found to have antirheumatic and sodium retaining effects which were about intermediary between those observed for analogues with para-methyl substitutions and those with meta-hydroxyl substitutions. On the other hand, G-32962, which has a meta-methyl group and a para-hydroxyl group, caused such severe gastrointestinal disturbance in dogs that further clinical studies were not warranted.

Studies were also carried out during the past year with several analogues which had changes in the pyrazolone ring structure of phenylbutazone. These compounds all had potent anti-inflammatory activity in animals but they were not absorbed sufficiently well for clinical studies.

(c) Mark difference exists in the renal excretion of various phenylbutazone analogues. For instance, phenylbutazone, Metabolite I and its p-methyl derivative are not excreted in any detectable amounts. However, Metabolite II and the sulfoxide derivative are excreted in fairly large amounts. Renal clearance studies have shown that the rapid urinary excretion of these compounds is due to their tubular secretion. It appears that only those analogues which are strongly acidic are secreted. Whether this observation bears any relationship to their potent uricosuric properties remains to be elucidated.

Significance to the Program of the Institute - A non-toxic potent antirheumatic drug would be of considerable value in the treatment of rheumatic fever.

Proposed Course of Project - (a) Up to the present we have mainly studied analogues of phenylbutazone which have substitutions in the benzene rings or butyl side chain. We are now investigating a series of drugs which have the butyl side chain replaced by various alkyl groups such as propyl, pentyl, and isobutyl. It is possible that such substitutions may yield a more ideal antirheumatic drug than phenylbutazone. In any case, information from such a study would essentially complete our documentation of a structural-activity relationship in the phenylbutazone series.

(b) Our studies have resulted in the introduction of two new drugs for the treatment of chronic tophaceous gout. One is a sulfoxide analogue (Anturan) and the other is Flexin. Although these compounds have potent uricosuric activity, they lack an antirheumatic effect. In addition both drugs are rapidly metabolized and thus must be administered to the patient at frequent intervals. The possibility that potent antirheumatic and uricosuric activity may be coupled together in the same drug was suggested from our observations with p-nitro phenylbutazone. For this reason several compounds have been studied. One of these, an α-keto analogue of Metabolite I, has been found by Dr. J. Seegmiller of N.I.A.M.D. to have potent uricosuric activity and also appears to possess some antirheumatic activity. Another compound is the para-hydroxyl analogue of G-25671 which is metabolized at a much slower rate than G-25671. Preliminary results indicate that a single dose of this drug exerts an exceptionally prolonged uricosuric effect in gouty patients.

(c) Evidence has been obtained for a metabolite of the sulfoxide which has a para-hydroxyl group in a benzene ring. Studies are now underway to establish this structure by comparison with reference compounds which have been synthesized for us.

(d) Gastric ulceration is one of the most serious side effects observed in patients receiving phenylbutazone. Recent studies now show that it is possible to produce ulceration in animals by giving large doses of the drug. Various analogues of phenylbutazone differ markedly in their ulcerogenic effect, thus affording a possible screening test for this side effect. Introduction of fluorine into the para positions of both benzene rings appears to enhance this ulcerogenic effect markedly. For instance, gastrointestinal bleeding was observed in dogs with this compound at a dose level where phenylbutazone was without effect. Further studies are planned in an attempt to relate ulcerogenic activity with chemical structure; such information would be of tremendous value in our search for a new antirheumatic drug.

(e) Phenylbutazone has a rather unique pattern of distribution in the body in that it is mainly localized in plasma. Preliminary results indicate that analogues which are more acidic than phenylbutazone (pK 4.5) are even more concentrated in plasma whereas those which are less acidic distribute preferentially into the tissues. It is planned to investigate this point with two analogues of Metabolite I which have widely different pK values: one is an α-keto derivative with pK of 2.0 and the other a tertiary butyl derivative with a pK 7.0.

Part B included Yes

Serial No. NHI-214

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Yü, T.F., Burns, J.J., Paton, B.C., Gutman, A.B. and Brodie, B.B.: Phenylbutazone metabolites: antirheumatic, sodium-retaining and uricosuric effects in man. J. Pharm. and Exptl. Thera. 123: 63-69, 1958.

Burns, J.J., Yü, T.F., Dayton, P., Berger, L., Gutman, A.B. and Brodie, B.B.: Relationship between pKa and uricosuric activity in phenylbutazone analogues. Nature, 182: 1162, 1958.

Yü, T.F., Burns, J.J. and Gutman, A.B.: A clinical trial with C-28315, a sulfoxide analogue of phenylbutazone, as a uricosuric agent in gouty subjects. Arthritis and Rheumatism, 1: 532-543, 1958.

Honors and Awards relating to this project:

None.

(b) Administration of various drugs to rats markedly stimulate the metabolism of glucose via the glucuronic acid pathway, as evident by enhanced urinary excretion of L-ascorbic acid. These drugs differ from one another in possessing completely different chemical and pharmacological properties and they include barbiturates, aminopyrine, antipyrine, phenylbutazone, Chloretone, Flexin, Dicipal, meprobamate, etc. Evidence has been obtained recently which suggests that the drugs may exert their effect by inducing the synthesis of certain enzymes involved in the glucuronic acid pathway. A clue to such a mechanism has come from the observation that various polycyclic hydrocarbons (3-methylcholanthrene, 1,2,5,6-dibenzanthracene, and 3,4-benzpyrene) markedly stimulate the synthesis of L-ascorbic acid. These hydrocarbons have been shown previously to induce the synthesis of specific liver microsomal enzymes which metabolize foreign compounds such as aminoazo dyes and polycyclic hydrocarbons. Several hydrocarbons which are inactive as inducers of synthesis of the enzymes which metabolize azo dyes have no effect on ascorbic acid synthesis. On the other hand, Chloretone and barbital, two potent stimulators of ascorbic acid excretion, also have distinct effect like the hydrocarbons in inducing the synthesis of the enzyme system which N-demethylates azo dyes. These observations suggest that a relationship may exist between the effect of foreign compounds on induced synthesis of microsomal enzymes and their effect on the glucuronic acid pathway. Barbital is of particular interest, for this compound is not metabolized but can exert these effects. It is tempting to speculate that these responses on the part of the body to foreign compounds may actually reflect a new "detoxification mechanism".

Significance to the Program of the Institute - Studies of factors regulating the synthesis of glucuronic acid and ascorbic acid may disclose a new detoxification mechanism to foreign compounds in the body.

Proposed Course of Project - (1) The effects of various drugs and other foreign compounds on the metabolism of glucose via the glucuronic acid pathway and on hepatic microsomal enzyme systems will be studied to determine if compounds which stimulate ascorbic acid biosynthesis also increase the activity of liver microsomal enzymes.

(2) The *in vitro* metabolism of D-glucose-C^{14} to D-glucuronic acid will be investigated with liver from normal and drug treated rats. Studies will be carried out to determine if drugs increase the activity of known enzymes of the glucuronic acid pathway.

(3) Hypophosectomy, but not adrenalectomy, markedly inhibits or completely prevents the stimulation of ascorbic acid excretion caused by drugs. Various pituitary hormones will be administered to hypophosectomized rats to determine if specific pituitary hormones are involved in the drug effect. Parallel experiments will also be carried out on the effect of pituitary hormones on the activity of liver microsomal enzymes.

Serial No. [illegible]-215

4. Studies will be carried out on the development of enzymes of the glucuronic acid pathway. The fetal activity of enzymes involved in biosynthesis of ascorbic acid and of microsomal enzymes which metabolize foreign compounds will be determined. The activity of these enzymes as well as the animals response to drugs as reflected by ascorbic acid excretion or by enhanced enzyme activity will be measured as a function of age from fetus to adult. These studies may give information on the mechanism of development of enzymes involved in the biosynthesis of ascorbic acid.

Part B included Yes

Serial No. NHI-215
1. Heart
2. Chemical Pharmacology
3. Clinical Pharmacology
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Studies on the Glucuronic Acid Pathway of Glucose Metabolism.

Principal Investigators: Dr. J.J. Burns
Dr. Allan Conney
Dr. Peter Dayton

Other Investigators: Miss Natalie Trousof
Miss Ruth Gastel
Mrs. Dolores Taller

Cooperating Unit: New York University Research Service, Goldwater Memorial Hospital, New York, New York.

Man Years (calendar year 1958):
Total: 1.36
Professional: .53
Other: .83

Patient Days (calendar year 1958): None.

Project Description:

Objectives - To study the occurrence in animals of the glucuronic acid pathway of glucose metabolism and to study the mechanism by which various drugs stimulate this metabolic pathway.

Methods Employed - A method has been developed for the determination of free glucuronic acid in urine. It involves ion-exchange chromatography followed by a specific reduction step with borohydride. Glucuronides and other urinary constituents which react with the naphthoresorcinol reagent do not interfere. This procedure has been of considerable help in following the excretion of free D-glucuronic acid after administration of certain drugs.

Patient Material - None.

Major Findings - (a) Studies on the biosynthesis of L-xylulose and L-ascorbic acid have pointed out a new pathway of glucose metabolism, the glucuronic acid pathway.
Mammalian tissues possess the enzymes for the various steps in this scheme. The occurrence of this pathway *in vivo* has now been confirmed in experiments in which labeled D-glucuronic acid and L-gulonic acid were found to be converted to glucose in accordance with the predictions of this scheme.

Serial No. MH-21

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Rosenberg, F., Dayton, P.G. and Burns, J.J.: Studies on the /Ascorbic acid pathway of glucose metabolism. J. Biol. Chem., in press.

Honors and Awards relating to this project:

None.

Serial No. NHI-216
1. Heart
2. Chemical Pharmacology
3. Organic Chemistry
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Isolation of Cardiotonic Substances from Mammalian Tissues.

Principal Investigator: Dr. Elwood O. Titus

Other Investigators: Mr. Herbert Spiegel
Mr. Roland Burroughs*
Dr. Stephen Hajdu*

Cooperating Units: *Laboratory of Kidney and Electrolyte Metabolism, NHI.

Man Years (calendar year 1958):
Total: 1.25
Professional: .25
Other: 1

Patient Days (calendar year 1958): None.

Project Description:

Objectives: Earlier reports have discussed evidence for the existence in mammalian tissues of substances that can exert digitalis like effects. It is the purpose of this project to isolate and identify such substances in the expectation that a knowledge of their structure may clarify their role, if any, in the functioning of the cardiovascular system.

In addition to lysolecithin, which has previously been reported to account for part of the cardiotonic activity of mammalian tissue extracts, there appear to be a number of lipoidal substances as yet unidentified. The immediate objective of this project is the characterization of these substances.

Methods Employed: The cardiotonic activity of tissue extracts is assayed in the isolated frog ventricle according to a procedure developed by Dr. Stephen Hajdu. This method depends upon the ability of digitalis like substances to prevent the decrease in contractile force which normally results from lengthening the intervals between stimuli. It offers considerable advantages in sensitivity and specificity and may be used for quantitative determinations of cardiac steroids.

The fractionation of tissue extracts is carried out for the most part by conventional chemical means. For the separation of very closely related, very non-polar lipids, chromatography on silicic acid has been most useful.

Patient Material: None.

Major Findings: Cardiotonic factors isolated from beef heart and from rabbit heart and blood have proved to be acidic lipids. From paper chromatographic data the beef heart substance appears to contain 18 to 20 carbon atoms. Infrared and ultraviolet spectra indicate an unsaturated, branched chain structure which owes its acidic properties to some functional group (possibly a β-diketone) other than the typical carboxyl of a fatty acid. Concentration of this factor is very low; one kilogram of beef heart contains an amount equivalent to 8 or 10 micrograms of strophanthidin.

Higher concentrations (equivalent to approximately 60 micrograms of strophanthidin per kilo) of active material occur in rabbit serum and heart. Preliminary chromatographic comparisons indicate that the heart and serum substances are probably identical. The serum factor appears to be a derivative of an α,β unsaturated acid containing at least 26 carbon atoms and one or more branched chain methyl groups. The substance can be obtained from freshly clotted blood, but not from plasma or from serum more than one or two hours after coagulation. Possible relationships between coagulation mechanisms and release of this substance remain to be explored. In the frog heart assay the substance is about as active as digitoxigenin and about one-twentieth as active as strophanthidin.

In addition to the above, a toxic acid from extracts of rabbit erythrocytes has been encountered. This factor induces sudden and irreversible contracture of the heart in a manner reminiscent of the action of saponins.

Significance to the Program of the Institute: Previous studies with the frog heart as well as reports from laboratories studying smooth muscle contraction indicate that minor structural variations may convert ordinarily innocuous lipids into substances with a variety of effects on muscular contraction. The effects on cardiac contractility reported here very probably reflect the influence of lipids on passage of inorganic ions through membranes to the contractile proteins.

These studies should therefore provide:

1. Data for theoretical studies of the role of lipids in membrane function.

2. A screening technique for determining which components of natural fats may be expected to have important effects on the cardiovascular system or on membrane function generally.

3. A means for detecting substances of mammalian origin which may have therapeutic possibilities in diseases of the cardiovascular system.

Proposed Course of Project: Efforts to characterize the active factors and studies of their possible role in coagulation mechanisms will continue.

Part B included No

Serial No. NHI-217
1. Heart
2. Chemical Pharmacology
3. Clinical Pharmacology
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Studies on the Metabolism of Sterols.

Principal Investigators: Dr. Elliott Schiffman
Dr. Elwood O. Titus

Other Investigator: None.

Cooperating Units: None.

Man Years (calendar year 1958):
Total: 1.25
Professional: 1.25
Other: None.

Patient Days (calendar year 1958): None.

Project Description

Objectives - Several routes of metabolism of cholesterol remain relatively unexplored. Of these the most interesting is the conversion of this sterol to the cardiac lactones, marinobufagin and marinobufotoxin, in the toad Bufo marinus.

Cholesterol and stigmasterol are metabolized to unknown steroids of increased polarity by Tetrahymena pyriformis, a protozoan. This conversion may be of considerable biochemical significance, since the growth inhibiting effects of carcinostatic purine antagonists upon this organism may be reversed by the presence of cholesterol or stigmasterol in the medium.

Methods Employed - Multiple chromatographic techniques have been used in the isolation and purification of compounds.

Various modifications of radio-autography have been employed in the assay of the results of tracer experiments.

Patient Material - None.

Major Findings - Research has proceeded in two directions:

(1) Attempts to find systems which efficiently synthesize the cardiostonic steroids.

a) In vivo experiments.

Mevalonic acid C^{14}, a very efficient sterol precursor, when injected into the toad caused an incorporation of isotope into the sterols, but not into the cardiac active substances.

ACTH was injected together with mevalonic acid. The hormone is known to stimulate the hydroxylation of steroids in the mammalian adrenal cortex. Since the conversion of sterols to cardiac lactones involve hydroxylation reactions, it was hoped that the hormone would increase the rates of such processes within the toad.

The results were as follows: an apparent stimulation of sterol synthesis from mevalonic acid was observed, but no appreciable effect was noted for cardiac lactone production.

b) In vitro experiments

Fortified toad gland homogenates in the presence of C^{14} substrates and ACTH showed no different synthetic activity from controls; nor did incubations of substrates with acetone powder systems with expressed venom mixtures provide any new information.

(2) The search for probable intermediates in the biosynthesis of lactones.

Previous experiments in this laboratory suggest that a rate limiting step in the overall synthesis of the cardiac lactones from cholesterol may be hydroxylation to the hitherto unknown 21-hydroxycholesterol. Since attempts to prepare this derivative with the aid of the 21-hydroxylating mold Ophiobolus herpotrichus were unsuccessful, a chemical synthesis from hyodesoxycholic acid has been devised. Several steps in this synthesis have been completed.

(3) It has recently been observed by Dr. Alexander Rich of the National Institute of Mental Health that desoxycholic acid can spontaneously form association complexes of very high molecular weight with an α-helical structure reminiscent of that of proteins. In order to complete the definition of the structure by X-ray diffraction, the hitherto unknown 3α, 12α, dihydroxy-20-bromo-20-norcholanic acid is required for use in the isomorphous replacement procedure. Since slight modification of the projected 21-hydroxy cholesterol synthesis offers a promising route to the latter compound, its synthesis has been undertaken in collaboration with Dr. Rich. Several steps have been completed.

Significance to the Program of the Institute - The metabolism of cholesterol in the systems described is of interest especially in the case of the cardiac active substances, the biosynthesis of which is relatively unexplored. It is hoped that information on this process will eventually contribute to understanding the mode of action of these substances at an enzymatic level.

Serial No. NHI-217

Proposed Course of Project - The attempt will be continued to synthesize the hydroxylated sterol, which could be an intermediate in the biological formation of the cardiac lactones.

Efforts are in progress to determine the nature of the cholesterol metabolites in tetrahymena.

In the search for systems that synthesize the lactones efficiently, the fate of isotopic β-sitosterol injected into the toad will be followed. This sterol is known to be present in the parotoid gland, which contains the cardiac lactones.

Part B included No

Serial No. NHI-218
1. Heart
2. Chemical Pharmacology
3. Organic Chemistry
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Study of the Properties of Phospholipase D.

Principal Investigators: Dr. Herbert Weiss
Dr. Elwood O. Titus

Other Investigator: None.

Cooperating Units: None.

Man Years (calendar year 1958):
Total: 1.25
Professional: 1.25
Other: None.

Patient Days (calendar year 1958): None.

Project Description:

Objectives - Phospholipase D is an enzyme that removes choline from lecithin and ethanolamine from cephalin. It has been found thus far only in plants but is presumed to be present in mammalian tissues, particularly since phosphatidic acids, which are products of its activity, have been recently found in normal mammalian tissues.

The purposes of this project are: purification of the enzyme, identification of the cofactors required for its activity, a search for its presence in animal tissues, and a study of its significance in the metabolism of phospholipids.

Methods Employed - Conventional methods of enzyme isolation were employed. Enzyme activity was measured by assay of liberated choline as the enneaiodide. Phospholipids were chromatographed on silicic acid.

Patient Material - None.

Major Findings - Highly purified fractions of phospholipase D prepared by ammonium sulfate fractionation require both calcium and one or more lipid activators found in soybean phospholipids. An activator isolated by solvent fractionation and repeated chromatography has been identified as phosphatidyl inositol.

The nature of the activating mechanism is obscure. It does not appear to involve solubilization of the substrate, since the lecithin of soluble egg lipoprotein (which is attacked by other phospholipases) is not hydrolyzed by this enzyme in the absence of activator.

Significance to the Program of the Institute - Lack of recognition of the peculiar cofactor requirements of phospholipase D may have prevented its identification in animal tissue. It is possible that this enzyme may be of importance in the metabolism of mammalian phospholipids.

Proposed Course of Project - Further studies of the mechanism of activation by phosphatidyl inositol will be carried out. A search for the enzyme and a study of its role in mammalian systems will be undertaken.

Part B included No

Serial No. NHI-219
1. Heart
2. Chemical Pharmacology
3. Clinical Pharmacology
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Studies with D-Ascorbic Acid

Principal Investigators: Dr. J.J. Burns
Dr. Peter G. Dayton

Other Investigator: Miss Constance Glascow (summer employee)

Cooperating Unit: Studies carried out in collaboration with Dr. H. Fullmer of the National Dental Institute.

Man Years (calendar year 1958):
Total: .28
Professional: .28
Other:

Patient Days (calendar year 1958): None.

Project Description:

Objectives - To study the Vitamin C activity of D-ascorbic acid. To contrast the physiological disposition of D-ascorbic acid and L-ascorbic acid in animals.

Patient Material - None.

Major Findings - Previous studies showed that D-ascorbic acid is excreted by scorbutic guinea pigs at a considerably more rapid rate than L-ascorbic acid which suggested a possible explanation for its reported lack of Vitamin C activity. In order to test this point, D-ascorbic acid was administered to scorbutic guinea pigs in such a way as to achieve concentrations in the body similar to those required for L-ascorbic acid. Under these conditions D-ascorbic acid cured certain but not all of the symptoms of scurvy. For instance, even in small doses this structural analogue was able to maintain the weight and survival of severely scorbutic guinea pigs. In addition, D-ascorbic acid was able to correct the defective dentine produced in scurvy much in the same way as L-ascorbic acid. However, despite the normal appearance of the scorbutic guinea pigs after treatment with D-ascorbic acid, they were found upon autopsy to have severe joint hemorrhages characteristic of the disease. Thus, the results show that D-ascorbic acid is able to replace L-ascorbic acid for some of the activities

of Vitamin C. This is of importance since it indicates a dual role for Vitamin C; one that is specific which requires only L-ascorbic acid, and the other that is non-specific in which D-ascorbic acid and perhaps other compounds with the same oxidation-reduction potential can substitute.

Significance to the Program of the Institute - Studies with D-ascorbic acid may furnish a clue to the action of L-ascorbic acid. It is well known that L-ascorbic acid is necessary for maintenance of connective tissue in the cardiovascular system.

Proposed Course of Project - (1) Further studies will be continued on the Vitamin C activity of D-ascorbic acid particularly in regard to formation of dentine. It is planned to compare the localization of D- and L-ascorbic acid-1-C^{14} by radioautographic techniques in this representative connective tissue.

(2) Experiments are planned to compare the excretion of D- and L-ascorbic acid by the kidney. Preliminary results indicate that D-ascorbic acid is not reabsorbed by the renal tubule cells like L-ascorbic acid. Studies along these lines may furnish information on mechanisms involved in the reabsorption of L-ascorbic acid.

(3) Little is known concerning the penetration of L-ascorbic acid across the blood brain barrier. This problem will be studied by contrasting the rate of penetration of D- and L-ascorbic acid into brain. Similar studies will also be carried out on the aqueous humor plasma barrier.

(4) Preliminary results show that D-ascorbic acid is not absorbed when given orally. Further experiments will be carried out to compare the absorption of D- and L-ascorbic acid. Such an approach may furnish a clue to the mechanism involved in the oral absorption of L-ascorbic acid.

Part B included Yes

(Attachment I)

Serial No. NHI-219

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Dayton, P.G. and Burns, J.J.: Metabolism of D-ascorbic acid-1-C^{14} in guinea pigs and rats. J. Biol. Chem. 231: 85-91, 1958.

Burns, J.J.: Vitamin C activity of D-ascorbic acid. Proc. of the IV International Congress of Biochemistry, Vienna, 1958, in press.

Honors and Awards relating to this project: None.

Serial No. NHI-220
1. Heart
2. Chemical Pharmacology
3. Clinical Pharmacology
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Studies with L-Ascorbic Acid.

Principal Investigators: Dr. John J. Burns
Dr. Peter G. Dayton
Dr. Allan Conney

Other Investigators: Mr. Julian Kanfer
Miss Carole Evans
Miss Ruth Gastel

Cooperating Unit: New York University Research Service, Goldwater Memorial Hospital, New York, New York.

Man Years (calendar year 1958):
Total: 2.03
Professional: .53
Other: 1.50

Patient Days (calendar year 1958): None.

Project Description:

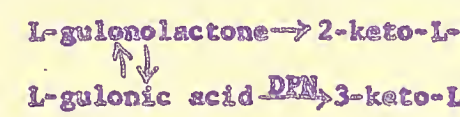
Objectives - To study the enzymes and intermediates involved in the biosynthesis and metabolism of L-ascorbic acid. To investigate the factors which control the physiological disposition of L-ascorbic acid.

Patient Material - None.

Major Findings - (a) Biosynthesis of L-ascorbic acid: L-Ascorbic acid is synthesized from glucose in rats as follows: D-Glucose→ D-glucuronic acid→L-gulonic acid→L-ascorbic acid. Man, monkey and guinea pig lack the enzyme required for the conversion of L-gulonic acid to L-ascorbic acid. It is this missing step which explains the need for Vitamin C in their diet to prevent scurvy.

During the past year information has been obtained which suggests that the mechanism for the synthesis of D-glucuronic acid occurs in the liver as follows: D-glucose→uridinediphosphoglucose→uridinedephosphoglucuronic acid→glucuronic acid. Confirmatory evidence for the importance *in vivo* of this pathway in the rat has come from finding that D-galactose-1-C^{14} is a considerably better precursor of D-glucuronic acid and L-ascorbic acid than is D-glucose-1-C^{14}.

The enzyme system in liver microsomes for the conversion of L-gulonic acid to L-ascorbic acid has been solubilized and partially purified. The lactone of L-gulonic acid is required as the substrate and no evidence was observed for a requirement of pyridine nucleotides. Studies with various structural analogues of L-gulonolactone and with model compounds have suggested 2-keto-L-gulonolactone as the intermediate. This system is entirely different than that in kidney which converts L-gulonic acid to L-xylulose. Based upon available evidence the following scheme is proposed for the formation of L-ascorbic acid and L-xylulose.



(b) Fate of L-ascorbic acid: Previous studies have shown that the major route of metabolism of L-ascorbic acid in vivo is by its almost complete oxidation to expired CO_2. A clue to the mechanisms involved has come from finding an active enzyme system in rat kidney which decarboxylates L-ascorbic acid through the intermediate formation of its oxidized products, dehydro-ascorbic acid and diketogulonic acid. Employing a partially purified system, the formation of a five carbon sugar acid has been detected, which has been identified as L-lyxonic acid.

Further in vivo studies were carried out to determine whether L-xylulose was an intermediate in the metabolism of the vitamin. The incorporation of carbon-6 labeled L-ascorbic acid, dehydro-ascorbic acid and diketogulonic acid into liver glycogen was measured. The results obtained exclude any appreciable metabolism via known pentoses but strongly suggest trioses as intermediates.

Significance to the Program of the Institute - L-ascorbic acid is necessary for the maintenance of connective tissue present in the cardiovascular system.

Proposed Course of Project - (1) Little is known concerning the biosynthesis of L-ascorbic acid in microorganisms. Preliminary studies show that D-glucuronic acid and L-gulonic acid, precursors of the vitamin in animals, are not converted to L-ascorbic acid in yeast. It is planned to investigate the metabolism of L-ascorbic acid in various representative microorganisms.

(2) Plants synthesize L-ascorbic acid from glucose by an entirely different mechanism than animals. Experiments will be carried out to determine whether animals lack the ability to make the vitamin by the plant pathway. It should be noted that birds and reptiles apparently have a different mechanism for synthesis of L-ascorbic acid than the rat in that the required enzymes are present in the kidney instead of liver.

(3) L-Ascorbic acid has a rather unique pattern of distribution in the body. It is concentrated in much greater amounts in such tissues as adrenals, pituitary and testes. In addition, it is found in connective tissues in essentially the same concentration as in the liver. Studies will be carried out to determine the mechanism of binding of the vitamin to tissue components.

(4) Further studies will be carried out to define the enzymatic mechanisms involved in the metabolism of L-ascorbic acid.

Part B included Yes

Serial No. NHI-220

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Burns, J.J., Kanfer, Julian and Dayton, P.G.: Metabolism of L-ascorbic acid in rat kidney. J. Biol. Chem. 232: 107-115, 1958.

Hellman, L. and Burns, J.J.: Metabolism of L-ascorbic acid-1-C^{14} in man. J. Biol. Chem. 230: 923-930, 1958.

Kanfer, J., Burns, J.J. and Ashwell, G.: L-Ascorbic acid synthesis in a soluble enzyme system from rat liver microsomes. Biochim. et Biophys. Acta, in press.

Dayton, P.G., Eisenberg, F., Jr., and Burns, J.J.: Metabolism of C^{14} labeled ascorbic dehydroascorbic and diketogulonic acids in guinea pigs. Arch. Biochem. Biophys., in press.

Burns, J.J. and Ashwell, G.: L-Ascorbic acid. in: Enzymes, Vol. II, in press.

Honors and awards related to this project:

None.

Serial No. NHI-221
1. Heart
2. Chemical Pharmacology
3. Clinical Pharmacology
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Studies with Muscular Relaxants.

Prinicpal Investigator: Dr. Allan Conney

Other Investigators: Miss Natalie Trousof
Dr. J. J. Burns

Cooperating Unit: New York University Research Service, Goldwater Memorial Hospital, New York, New York.

Man Years (calendar year 1958):
Total: .69
Professional: .36
Other: .33

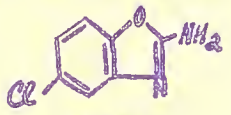
Patient Days (calendar year 1958): None.

Project Description:

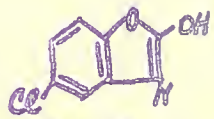
Objectives - To study the metabolism in man of the two new muscular relaxants of the benzoxazole series, Flexin and Paraflex.

Patient Material - None.

Major Findings: Flexin has been used for the past several years as a muscular relaxant drug which is thought to act centrally like mephenesin. The drug is converted in man to a metabolite formed by substitution of a hydroxyl group for the amino group. This metabolite has potent muscular relaxant activity, and it has recently been introduced for this purpose as a new drug, Paraflex. Further studies have shown that the major route of metabolism of both Flexin and Paraflex in man is by introduction of a hydroxyl group into the benzene ring of each compound. The overall scheme for the metabolism of Flexin and Paraflex is as follows:

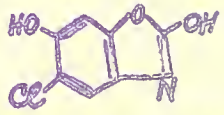


Flexin



Paraflex

Hydroxy-Flexin



Hydroxy-Paraflex

Neither hydroxy-Flexin nor hydroxy-Paraflex has muscular relaxant activity in animals even at high doses. As pointed out in another report, Flexin possesses potent uricosuric activity in gouty patients, but its two metabolites, Paraflex and hydroxy-Flexin, do not possess this activity.

Flexin and Paraflex are enzymatically hydroxylated in vitro by liver homogenate fortified with TPN^{+} and glucose-6-phosphate. The enzyme systems catalyzing these reactions are similar to a group of microsomal TPNH requiring enzymes which oxidize a variety of foreign compounds.

Significance to the Program of the Institute - There is considerable need in clinical medicine for an effective muscular relaxant for the treatment of multiple sclerosis, cerebral palsy, poliomyelitis and other diseases associated with peripheral muscular spasms.

Proposed Course of Project - No further work is planned on this project. Two manuscripts are now in preparation which will be submitted shortly to the American Journal of Pharmacology and Experimental Therapeutics.

Part B included No

Serial No. NHI-222
1. Heart
2. Chemical Pharmacology
3. Clinical Pharmacology
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Studies with L-Xylulose.

Principal Investigator: Dr. John J. Burns

Other Investigator: Mr. Julian Kanfer

Cooperating Units: Studies carried out in collaboration with Dr. Gilbert Ashwell of the National Institute of Arthritis and Metabolic Diseases.

Man Years (calendar year 1958):
Total: .61
Professional: .11
Other: .50

Patient Days (calendar year 1958): None.

Project Description:

Objectives - To study the origin and fate of L-xylulose, the sugar excreted by patients with essential pentosuria.

Patient Material - None.

Major Findings - Previously we described an active enzyme in rat kidney which decarboxylates L-gulonic acid forming L-xylulose. The enzymes, L-gulonic acid (DPN) dehydrogenase has now been purified about 35-fold from hog kidney and a study of its properties was undertaken. Evidence has been obtained for 3-keto-L-gulonic acid as the intermediate in the reaction.

Significance to the Program of the Institute - L-Xylulose is an intermediate in a new pathway of glucose metabolism present in animal tissues.

Proposed Course of Project - (1) In the course of the isolation of L-xylulose another metabolic product was uncovered which has completely different properties from any known pentose. The identification of this compound and its mechanism of formation will be studied.

(2) A small amount of L-ascorbic acid was observed to be formed from L-gulonic acid in the kidney system. The possibility that this may reflect a different mechanism of L-ascorbic biosynthesis than that found in liver will be investigated.

Part B included Yes

(Attachment 1)

Serial No. NHI-222

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Kanfer, J.: Formation of L-xylulose from L-gulonolactone in rat kidney. Dissertation for the Degree of Master of Science, George Washington University, 1958.

Ashwell, G., Kanfer, J. and Burns, J.J.: Stuxies on the mechanism of L-xylulose formation by kidney enzymes. J. Biol. Chem., in press.

Honors and Awards relating to this project: None.

Serial No. NHI-223
1. Heart
2. Chemical Pharmacology
3. Clinical Pharmacology
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Studies with Inositol.

Principal Investigator: Dr. John J. Burns

Other Investigators: Miss Natalie Trousof
Miss Carole Evans
Dr. Nicholas Papadopoulos

Cooperating Unit: This study was carried out in collaboration with Dr. Bernard W. Agranoff of the National Institute of Neurological Diseases and Blindness.

Man Years (calendar year 1958):
Total: .94
Professional: .11
Other: .83

Patient Days (calendar year 1958): None

Project Description:

Objectives - To investigate the precursor role of inositol for the biosynthesis of D-glucuronic acid, L-gulonic acid and L-ascorbic acid.

Patient Material - None.

Major Findings - Evidence has been presented by others that inositol is converted to a racemic mixture of D,L-glucuronic acid by enzymes in rat kidney. Since D-glucuronic acid can serve as a precursor of L-gulonic acid and L-ascorbic acid, the role of inositol in this biosynthetic pathway was studied with inositol-H^3 and inositol-C^{14}. The results obtained indicate that inositol is converted in the rat to D-glucuronic acid and L-gulonic acid. Since previous studies have shown that these sugar acids are degraded in the body via L-xylulose it is now apparent that pentoses play an important role in the metabolism of inositol. No conversion of inositol to L-ascorbic acid or L-glucuronic acid was detected in the rat.

Significance to the Program of the Institute - Inositol is a constituent of various phospholipids which are important in the biochemistry of the brain, heart, liver and other organs.

Proposed Course of Project - This project is completed.

Part B included Yes

(Attachment I)

Serial No. NHI-223

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Burns, J.J., Trousof, N., Evans, C., Panadopoulos, N., and Agranoff, B.W.: Conversion of Myo-inositol to D-glucuronic acid and L-gulonic acid in the rat. Biochim. et Biophys. Acta, in press.

Honors and Awards relating to this project: None.

Serial No. NHI-224
1. Heart
2. Chemical Pharmacology
3. Clinical Pharmacology
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Uricosuric Effect of Flexin.

Principal Investigator: Dr. J. J. Burns

Other Investigators: None

Cooperating Units: Dr. Alexander B. Gutman and Dr. T.F. Yü, Mount Sinai Hospital, New York and New York University Research Service, Goldwater Memorial Hospital, New York, New York.

Man Years (calendar year 1958):
Total: .11
Professional: .11
Other: None.

Patient Days (calendar year 1958): None.

Project Description:

Objectives - To investigate the effect of Flexin and related benzoxazole derivative on uric acid excretion.

Patient Material - None.

Major Findings - Flexin (zoxazolamine) has been widely used as a muscle relaxant for several years. While studying the biochemical fate of Flexin in the body it was found that relatively large amounts of a crystalline compound was excreted in the urine of patients who had received the drug. Although the crystals at first were thought to be a metabolic product of Flexin, they proved to be uric acid, and it was this observation that gave the first clue to the muscle relaxants potent uricosuric properties. The drug has a considerably greater uricosuric effect than any other currently available. Our observation has stimulated its clinical trials by rheumatologists throughout the country. It is expected that Flexin will be introduced as a new drug for the treatment of gout in the near future.

Significance to the Program of the Institute - Studies on how drugs effect uric acid excretion increase our general knowledge on the mechanisms by which various naturally occurring compounds are excreted by the kidney.

Proposed Course of Project - (1) The clinical evaluation of Flexin as a uricosuric agent in the treatment of chronic gout will be continued. In particular the drug will be tested in gouty patients that are refractory to probenecid.

(2) Flexin has a chemical structure entirely different from that of other known uricosuric agents. The drug is a weak base whereas probenecid, the various phenylbutazone analogues, and salicylates are all strongly acidic compounds. The possibility that Flexin is exerting its uricosuric effect by an entirely different mechanism will be investigated.

(3) Studies are underway to determine the structural features in the Flexin molecule required for its uricosuric activity. Various compounds are being synthesized by McNeil Laboratories, Philadelphia, and will be tested for uricosuric activity. So far it has been found that a metabolite of the drug (Paraflex) which is formed by replacement of the amino for an hydroxyl group has no uricosuric activity. Another metabolite in which a hydroxyl group is introduced into the benzene ring is also inactive. However, a bromo substituted derivative does have activity but of a lower order than Flexin. It is hoped that studies in this direction will give a lead towards finding an even better uricosuric agent than Flexin. Such a compound would be one that is metabolized at a slower rate than Flexin; thus making unnecessary frequent medication.

Part B included Yes

Serial No. NHI-27.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Burns, J.J., Yü, T.F., Berger, Lawrence, and Gutman, Alexander B.: Zoxazolamine, Am. J. Med. 25: 401 408, 1958.

Honors and Awards relating to this project:

None.

Serial No. NHI-225
1. Heart
2. Chemical Pharmacology
3. Clinical Pharmacology
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

PART A

Project Title: Studies with Anticoagulants.

Principal Investigator: Dr. Peter G. Dayton

Other Investigator: None.

Cooperating Unit: Drs. Murray Weiner and Theodore Chenkin, New York University Research Service, Goldwater Memorial Hospital, New York.

Man Years (calendar year 1958):
Total: .17
Professional: .17
Other: None.

Patient Days (calendar year 1958): None.

Project Description:

Objectives - The effect of coumarin anticoagulants on blood coagulation of normal, scorbutic, starved and Vitamin K treated guinea pigs will be studied.

Methods Employed - Besides prothrombin times and recalcification times, the plasmas will be studied in the thromboelastograph (coagulograph). This instrument measures clot firmness.

Patient Material - None.

Major Findings - The project has just been initiated.

Significance to the Program of the Institute - Coumarin anticoagulant drugs are of considerable importance in the treatment of heart disease associated with the formation of small clots within the blood vessels. Information obtained from this program may aid in an understanding of the mode of action of these drugs.

Proposed Course of Project - It has been observed by Link and coworkers that ascorbic acid reduced the effectiveness of a particular dose of dicumarol; on the other hand, scorbutic guinea pigs were more sensitive to dicumarol than normal animals. These experiments were carried out at a time where dicumarol was the only available coumarin anticoagulant. This drug, because of its insolubility is poorly absorbed or when given parenterally, has to be given in strongly alkaline solution. Therefore, it is not surprising that Link (who used

orally administered dicumarol) obtained variable results. At present, highly active soluble coumarin anticoagulants are available. In the proposed studies it is planned to use sodium acenocoumarin (Sintrom), which falls in this category and can be injected at neutral pH. It is hoped that by repeating and extending Link's studies under more reproducible conditions, information will be gained on the effect of ascorbic acid on the response of guinea pigs to coumarin anticoagulants.

The variability in the clinical response to coumarin anticoagulants appears to be due in part to nutritional factors. Besides ascorbic acid, Vitamin K is likely to play a role in such cases. Therefore, it is planned to study the effect of exogenous Vitamin K on the response of guinea pigs to acenocoumarin. It is hoped that from these animal experiments a lead may be found which will indicate how to carry out conclusive studies in man on the importance of nutritional status in coumarin anticoagulant therapy; in other words, on prothrombin synthesis.

Part B included No

Form No. ORP-2
Oct. 1957

PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
Laboratory of Clinical Biochemistry

Estimated Obligations for FY 1959

Total: $205,320

Direct: $156,000

Reimbursements: $ 49,320

Serial No. NHI-226
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies on Serotonin Metabolism.

Principal Investigator: Sidney Udenfriend

Other Investigator: Betty G. Redfield

Cooperating Units:
Dr. A. Sjoerdsma, Section of Experimental Therap., NHI
Dr. S. Hess, Laboratory of Chemical Pharmacology, NHI
Dr. B. Witkop, Laboratory of Chemistry, NIAMD
Mr. W. Lovenberg, United Fruit Company

Man Years:
Total: 0.83
Professional: 0.83
Other:

Patient Days:

Project Description:

It was previously shown that inhibitors of the enzyme, monoamine oxidase (MAO), would not prevent the metabolism of parenterally administered serotonin. In animals it is now evident that an alternate route of metabolism leading to serotonin-O-glucuronide efficiently metabolizes parenteral serotonin when MAO is blocked. Serotonin-O-glucuronide was identified by enzymatic methods.

In man too, MAO inhibitors do not have much of an effect on the metabolism of ingested serotonin. However, no serotonin glucuronide could be detected in human urine. In preliminary experiments with Dr. Sjoerdsma, C^{14} labelled serotonin was administered to patients who had been receiving JB-516. A major metabolite appeared which remains to be identified. In agreement with these chemical findings Dr. Leon Goldberg has shown that MAO inhibitors do not appreciably potentiate parenteral serotonin.

In contrast, it has been shown that the metabolism of endogenous serotonin, in brain is markedly inhibited. Following administration of harmaline brain serotonin levels rise very rapidly suggesting a possible turnover time of minutes. This compares to turnover times of hours in peripheral serotonin depots.

It has also been shown conclusively that tryptamine is not converted to serotonin. This was done with labelled tryptamine in living rabbits.

Methods were developed for measuring the harmala alkaloids, potent and reversible MAO inhibitors, in tissues and urine. The reversible nature of the inhibition was shown both *in vitro* and *in vivo*. Studies with these inhibitors in man (with Dr. A. Sjoerdsma) suggested that they are not readily absorbed from the GI tract. Experiments in animals seem to confirm this poor absorption.

Additional screening for reversible MAO inhibitors has continued and several unsuspected groups have been uncovered.

procaine amide and analogues (Squibb)
orthoxine (Upjohn)

Both of these are more active than marsilid, *in vitro*. However, they have little *in vivo* activity.

It was shown that bananas contain large amounts of serotonin, 20-40 γ/gram in the pulp, which gives rise to 5-hydroxyindoleacetic acid on ingestion (see clinical report by Dr. A. Sjoerdsma). Serotonin is also found in other fruits in smaller amounts (5-10 γ/gram); these include tomatoes, plums, avocadoes, and egg plant. Other amines have also been found in the banana including dopamine, noradrenaline and tyramine.

Further studies on the isolation and enzymatic formation of serotonin-O-glucuronide will continue. The nature of the alternate pathway in man will also be investigated. Attempts will be made to develop harmala alkaloids which will be more readily absorbed from the GI tract. Purification of MAO will be undertaken to permit studies on cofactors and mechanism of action. Other reversible inhibitors of MAO and of 5-hydroxytryptophan decarboxylase will be investigated. Work, including enzymatic studies, will continue on bananas and other fruits.

Part B included: Yes

Serial No. NHI-226

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Publications

1. Weissbach, H., Redfield, B.G., and Udenfriend, S. Soluble Monoamine Oxidase: Its Properties and Actions on Serotonin. J. Biol. Chem. 229: 953-963, 1957.

2. Weissbach, H., Bogdanski, D.F., and Udenfriend, S. Binding of Serotonin and Other Amines by Blood Platelets. Arch. Biochem. and Biophys. 73: 492-499, 1958.

3. Weissbach, H., Waalkes, T.P., and Udenfriend, S. A Simplified Method for Measuring Serotonin in Tissues; Simultaneous Assay of Both Serotonin and Histamine. J. Biol. Chem. 230: 865-871, 1958.

4. Freter, K., Weissbach, H., Redfield, B.G., Udenfriend, S., and Witkop, B. Oxyindole Analogues of (5-Hydroxy) Tryptamine and Tryptophan, as Inhibitors of the Biosynthesis and Breakdown of Serotonin. J. Amer. Chem. Soc. 80: 983-987, 1958.

5. Bogdanski, D.F., Weissbach, H., and Udenfriend, S. Pharmacological Studies with the Serotonin Precursor, 5-Hydroxytryptophan. J. Pharm. and Exp. Therap. 122: 182-194, 1958.

6. Udenfriend, S., Weissbach, H., and Brodie, B.B. Assay of Serotonin and Related Metabolites, Enzymes, and Drugs. Methods of Biochemical Analysis Vol. VI, New York, Interscience Publishers Inc., pages 95-130, 1958.

7. Udenfriend, S., and Weissbach, H. Turnover of 5-Hydroxytryptamine (Serotonin) in Tissues. Proc. Soc. Exp. Biol. and Med. 97: 748-751, 1958.

8. Waalkes, T.P., Sjoerdsma, A., Creveling, C.R., Weissbach, H., and Udenfriend, S. Serotonin, Norepinephrine and Related Compounds in Bananas. Science 127: 648-650, 1958.

9. Udenfriend, S. Metabolism of 5-Hydroxytryptamine. 5-Hydroxytryptamine, London, Pergamon Press, pages 43-49, 1958.

10. Sjoerdsma, A., Gillespie, L., and Udenfriend, S. A Simple Method for the Measurement of Monoamine Oxidase Inhibition in Man. Lancet 2: 159, 1958.

11. Hess, S., Weissbach, H., Redfield, B.G., and Udenfriend, S. The Relationship Between Iproniazid Metabolism and the Duration of its Effect on Monoamine Oxidase. J. Pharm. and Exp. Therap. 124: 189-193, 1958.

12. Udenfriend, S., Witkop, B., Redfield, B.G., and Weissbach, H. Studies with Reversible Inhibitors of Monoamine Oxidase. Harmaline and Related Compounds. Biochem. Pharmacol. In Press.

Serial No. NHI-227
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Indoleacetic Acid and Tryptamine

Principal Investigator: John B. Jepson

Other Investigators: Betty G. Redfield and Chozo Mitoma

Cooperating Units:
Dr. A. Sjoerdsma, Section of Experimental Therap., NHI
Dr. J. Oates, Section of Experimental Therap., NHI
Drs. B. Witkop and J. Daly, Laboratory of Chemistry, NIAMD

Man Years:
Total: 1.33
Professional: 1.33
Other:

Patient Days:

Project Description:

This project started as an investigation of the intermediates involved in the biogenesis of indoleacetic acid (IAA) in animals and man. It is now apparent that the bulk of IAA arises through transamination of tryptophan to the keto acid followed by decarboxylation. However, it has been shown that tryptamine can arise in animal, plant and bacterial tissues.

Tryptophan decarboxylase has been demonstrated in mammalian tissues and tryptamine itself has been demonstrated in urine and tissues. Procedures for assay of tryptamine have been developed.

It has been shown that tryptamine is not converted to serotonin. However, it is hydroxylated to 7-hydroxytryptamine, first shown by Japanese investigators and now corroborated in this laboratory. When monoamine oxidase (MAO) inhibitors are administered the tryptamine levels in tissues are increased (brain from ≤ 0.3 to 1.0 γ/gram, liver from 0.6 to 2.0 γ/gram). When both tryptophan and MAO inhibitors are administered the increases in tryptamine are even greater. MAO inhibitors also increase excretion of urinary tryptamine. In studies with Dr. Sjoerdsma and his colleagues increases in urinary tryptamine were shown to be the earliest signs of MAO inhibition in man.

Preliminary studies indicate that reserpine releases tryptamine from tissues in the same way it releases serotonin, noradrenaline and dopamine.

Tryptamine has been found in many fruits including tomato. It is not present in bananas.

S. faecalis can decarboxylate tryptophan and the decarboxylase has been under study.

Both animal and bacterial tryptophan decarboxylase will be further purified and characterized.

More sensitive and specific methods for tryptamine will be developed.

Tryptamine metabolism will be studied in vivo and in vitro, including 7-hydroxylation, and possible conversion to harmala type alkaloids.

Relationship of tryptophan nutrition to amine metabolism, with and without MAO inhibitors, will be investigated.

Part B included: Yes

Serial No. NHI-227

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Publications

1. Weissbach, H., King, W., Sjoerdsma, A. and Udenfriend, S. Formation of Indole-3-Acetic Acid and Tryptamine in Animals. A Method for Estimation of Indole-3-Acetic Acid in Tissues. J. Biol. Chem. In Press.

Serial No. NHI-228
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Biogenesis and Metabolism of Hydroxyproline and Hydroxylysine

Principal Investigator: Chozo Mitoma

Other Investigators: Thomas E. Smith, Frances DaCosta, and Sven Lindstedt

Cooperating Units:
Drs. A. Sjoerdsma and S. Prockop, Section of Experimental Therap., NHI

Man Years:
Total: 2.83
Professional: 0.83
Other: 2

Patient Days:

Project Description:

Although free proline is a better precursor than free hydroxyproline for the bound hydroxyproline, it has been established that in rapidly growing chick embryos, an appreciable amount of hydroxyproline is directly incorporated into collagenous tissues.

Preliminary studies on the conversion of proline to hydroxyproline in a cell free system indicate that the product may be a conjugated form of hydroxyproline.

Ketoproline, an analogue of hydroxyproline, causes a prolonged elevation of free hydroxyproline in chick embryos and in the blood of rats. The mechanism for this elevation has been established to be due to (a) inhibition of hydroxyproline catabolism by ketoproline and (b) enzymatic conversion of ketoproline to hydroxyproline. The enzyme for the latter reaction is found in the supernatant fraction of rat kidney and liver and requires reduced pyridine nucleotide.

Among the various groups of patients studied, only those with Marfan's syndrome consistently excreted a higher amount of hydroxyproline than did normals. The total amino acid excretion by these patients is, however, within the normal range. This increased excretion of hydroxyproline is not reduced by putting the patient on a hydroxyproline free diet for two weeks.

The mechanism and the catalysts involved in the conversion of proline to hydroxyproline will be investigated. Similar studies will be conducted with hydroxylysine.

The effect of prolonged administration of ketoproline to animals will be studied. The enzyme involved in the conversion of ketoproline to hydroxyproline will be purified and studied.

The possibility of a more rapid turnover of collagen in patients with Marfan's syndrome than in normals will be investigated using labeled proline or hydroxyproline.

The metabolism of hydroxylysine in animals and particularly in micro-organisms will be studied. A phospatide containing this amino acid has been reported to occur in some species of bacteria.

Part B included: Yes

Serial No. NHI-228

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Publications

1. Mitoma, C., Smith, T.E., Friedberg, F., and Rayford, C. Incorporation of Hydroxyproline into Tissue Proteins by Chick Embryos. J. Biol. Chem. In Press.

2. Mitoma, C., Smith, T.E., DaCosta, F.M., Udenfriend, S., Patchett, A.A., and Witkop, B. Studies on 4-Keto-L-Proline. Science. In Press.

3. Mitoma, C., Smith, T.E., Davidson, J.D., Udenfriend, S., DaCosta, F.M., and Sjoerdsma, A. Improvements in Methods for Measuring Hydroxyproline: Application to Human Urine. J. Lab. and Clin. Med. In Press.

Serial No. NHI-229
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies on Adrenaline, Noradrenaline and Related Catechol Compounds

Principal Investigator: Sidney Udenfriend

Other Investigators: Cyrus R. Creveling and Lemuel C. Ieeper

Cooperating Units:
Drs. B. Witkop and M. Ozaki, Laboratory of Chemistry, NIAMD
Dr. A. Sjoerdsma, Section of Experimental Therap., NHI

Man Years:
Total: 1.83
Professional: 0.33
Other: 1.5

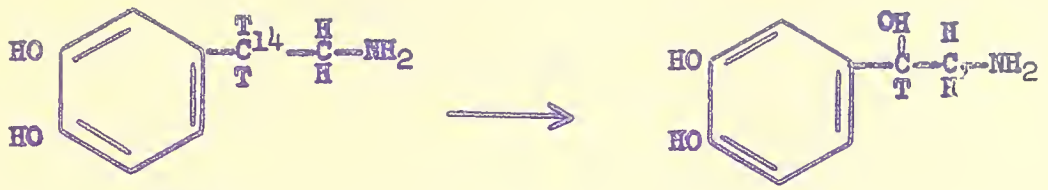
Patient Days:

Project Description:

The conversion of 3,4-dihydroxyphenylethylamine (dopamine) to noradrenaline has been studied intensively. It has been found that this reaction can be catalyzed by brain homogenates. Furthermore the catalyst is localized in brain stem areas, being very highly concentrated in hypothalamus and caudate nucleus and absent from cortex and cerebellum. The activity in the active areas surpasses that found in adrenal medulla.

Using brain enzymes it has been possible to elucidate the mechanisms of this side chain hydroxylation. Studies by Dr. Witkop demonstrated that an intermediate quinone methine could yield noradrenaline:

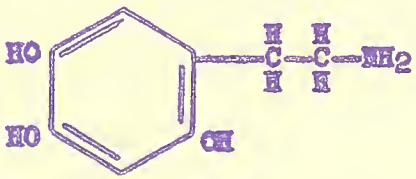
The chemical reaction and the enzymatic reaction were compared using double labeling procedures with tritium and C^{14} as follows:



dopamine

It was found that only one tritium is lost during the conversion of dopamine to noradrenaline both chemically and enzymatically. This and other considerations indicate that the reaction does not take place by a simple dehydrogenation and hydration of the side chain as is true for most instances of alcohol formation.

An interesting finding resulting from these studies was that a product of dopamine autoxidation may also be formed when dopamine is administered to animals.



3,4,6-Trihydroxyphenylethylamine

Methods have been devised to study noradrenaline metabolism *in vivo* without using isotopes. With this procedure it has been shown that potent monoamine oxidase inhibitors do not influence metabolism of parenteral noradrenaline. In agreement with Axelrod the bulk of such metabolism occurs via O-methylation. Preliminary studies indicate that compounds which are metabolized by methylation may block the metabolism of parenterally administered noradrenaline.

Studies will continue on the conversion of dopamine to noradrenaline. Methods will be developed for 3,4,6-trihydroxyphenylethylamine and its *in vivo* significance will be investigated.

Further attempts will be made to find inhibitors of O-methylation.

Serial No. NHI-229

PHS-NIH
Individual Project Report
Calendar Year 1958

Part B: Publications

1. Rosenfeld, G., Leeper, L.C., and Udenfriend, S. Biosynthesis of Norepinephrine and Epinephrine by the Isolated Perfused Calf Adrenal. Arch. Biochem. and Biophys. 74: 252-265, 1958.

2. Sjoerdsma, A., King, W., Leeper, L.C., and Udenfriend, S. Demonstration of the 3-Methoxy Analogue of Norepinephrine in Man. Science 127: 876, 1958.

3. Leeper, L.C., Weissbach, H., and Udenfriend, S. Studies on the Metabolism of Norepinephrine, Epinephrine and Their O-Methyl Analogues by Partially Purified Enzyme Preparations. Arch. Biochem. and Biophys. 77: 417-427, 1958.

4. Sjoerdsma, A., Leeper, L.C., Terry, L.L., and Udenfriend, S. Studies on the Biogenesis and Metabolism of Norepinephrine in Patients with Pheochromocytoma. J. Clin. Invest. In Press.

Serial No. NHI-230
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Transport of α-Amino Acids In Vivo

Principal Investigator: Sidney Udenfriend

Other Investigator: None

Cooperating Units:
Drs. M. Chirigos and P. Greengard, Geigy Pharmaceutical Corp.

Man Years:
Total: 0.33
Professional: 0.33
Other:

Patient Days:

Project Description:

During studies with the serotonin precursor, 5-hydroxytryptophan (5HTP), it became evident that this amino acid penetrated into the central nervous system (CNS) with great rapidity. Studies with other amino acids o-tyrosine and m-tyrosine indicated that these also penetrated with great ease. Following these observations studies were extended to other amino acids including many of the naturally occurring ones. It is now apparent that the L amino acids can be taken up by the CNS in a manner analogous to the uptake by other cells. Thus neutral L-amino acids are readily taken up, acidic ones are not. L-Amino acids are taken up better than are the corresponding D amino acids.

The term blood-brain barrier is a pharmacological phrase to explain differences in penetration of foreign substances into brain and other tissues. Although such an apparent barrier may exist for drugs it is obvious that not only is there no barrier to essential nutrients but that these are actively taken up by the brain. These studies on amino acid transport will not only provide valuable fundamental information but may yield practical results too. Well known drugs with amino acid side chains will be investigated to determine the effects of such substitution on their penetrability into brain and on their pharmacological actions.

These studies will be extended using a variety of amino acids and their congeners. The mechanism of penetration of amino acids into brain will be carefully investigated, as to cofactors, competition by other amino acids, etc.

Methods for determining an L amino acid in the presence of its D isomer has made it possible to investigate racemization in vivo. Such studies will be carried on.

Part B included: No

Serial No. NHI-231
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Chemical Assay of 3-Methoxy Adrenaline, 3-Methoxy Noradrenaline and 3-Methoxy-4-Hydroxymandelic Acid, Metabolites of Adrenaline and Noradrenaline

Principal Investigator: John J. Pisano

Other Investigators: None

Cooperating Units:
Dr. A. Sjoerdsma and Mr. E. Marsh, Section of Experimental Therap., NHI

Man Years:
Total: 0.33
Professional: 0.33
Other:

Patient Days:

Project Description:

3-Methoxy adrenaline and noradrenaline and 3-methoxy-4-hydroxy-mandelic acid have recently been shown to be the major metabolites of adrenaline and noradrenaline in man. Chemical assay for these metabolites would yield valuable information on the metabolism of these two important hormones in health and disease as well as yield information on the action of many drugs.

3-Methoxy adrenaline and noradrenaline have been quantitatively recovered from urine by adsorbing on Dowex 50-collidine, elution from the column with NH_4Cl and colorimetric assay of the eluate with chloro-imide. Normal urine controls give little or no color and as little as 2 µg/ml of the metabolites could be determined. Normal whole mice also give little or no color indicating the rather high degree of specificity obtained with the Dowex 50-collidine column. Urines from 6 patients with pheochromocytoma were 50 per cent positive.

Recent reports have shown that the excretion of the above two metabolites occurs mainly as a bound, easily hydrolyzable form. This might account in part for the results with the "pheo" urine.

Hydrolyzing the urine is essential to complete analysis of 3-methoxy adrenaline and noradrenaline. Unfortunately both these compounds are unstable to weak acid hydrolysis and must be protected. Preliminary results indicate that protection may be afforded by hydrolyzing in the presence of Dowex 50 (H^+). This resin has a high affinity for 3-methoxy

adrenaline and noradrenaline and is entirely stable during the hydrolysis. What happens, in effect, is that as the bound metabolites are liberated during hydrolysis they immediately get out of solution by adsorbing on the insoluble resin from which they can be subsequently easily removed.

Assay for 3-methoxy-4-hydroxymandelic acid could not be accomplished with an ion exchange resin because of the widespread occurrence of many phenolic acids which would behave as the metabolite on the resins and in colorimetric assays. One distinctive part of the molecule is the alcoholic group next to the benzene ring. Such an alcoholic group can be easily oxidized to a ketone which would show a high absorbtion in the ultraviolet. Using MnO_2 as the oxidant a strong peak at 350 mμ was obtained and as little as 1.5 μg/ml of 3-methoxy-4-hydroxymandelic acid could be determined in pure solution. When applied to urine several problems arose which have not been completely solved.

Present research is directed (1) to obtaining quantitative recovery of 3-methoxy-4-hydroxymandelic acid in urine. The approach to be taken is purification of urine by separation of the phenolic acid and use of larger quantities of MnO_2 or other oxidants, (2) to determine the efficiency of Dowex 50 (H^+) in protecting 3-methoxy adrenaline and noradrenaline from acid hydrolysis, and (3) to study the excretion of these metabolites in health and disease and in various drug treatments.

Part B included: No

Serial No. NHI-232
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Biosynthesis and Metabolism of γ-Guanidinobutyric Acid and γ-Aminobutyric Acid

Principal Investigator: John J. Pisano

Other Investigator: David Abraham

Cooperating Units:
Dr. Harry Grundfest, Columbia University Medical School, New York

Man Years:
Total: 1.33
Professional: 0.33
Other: 1

Patient Days:

Project Description:

The biosynthesis of γ-guanidinobutyric acid has been shown to occur in brain and retina of all species tested and most actively in dog pancreas in addition to rat kidney, as reported last year. Due to the current interest in γ-aminobutyric acid as a central synaptic inhibitor, collaboration with Dr. H. Grundfest of Columbia University was initiated with the result that γ-guanidinobutyric acid was found to also be a potent cortical synaptic inhibitor. It is interesting that the mechanism of inhibition is different from γ-aminobutyric. The physiological significance of this reaction is unknown. Assay of various tissues has revealed the widespread occurrence of γ-guanidinobutyric acid with brain having higher than average levels. Injection of γ-guanidinobutyric acid in rats was not accompanied by any increase in brain indicating the inability of γ-guanidinobutyric acid to pass the blood brain barrier.

Transamidinase is the enzyme which catalyzed the synthesis of γ-guanidinobutyric acid in mammals. The oxidation and decarboxylation of arginine is the pathway in many invertebrates and turkey liver. This oxidation pathway could not be demonstrated in the rat. Transamidinase can also catalyze the synthesis of δ-guanidinovaleric acid from δ-aminovaleric acid. It is interesting that the former compound has recently been reported to occur in human urine.

A new enzyme has been discovered in rabbit liver and kidney which hydrolyzes γ-guanidinobutyric acid to form γ-aminobutyric acid in urea. This enzyme is not arginase but might be identical to "hetero-arginase" reported some time ago to occur in rabbit intestinal mucosa.

Guinea pig liver is also active but distinction from arginase has not yet been established in this tissue. Heart, kidney and intestines of guinea pig were negative. Rat liver was also negative.

Current research is directed towards (1) the purification and significance of the new enzyme of rabbit liver and kidney, (2) the significance of transamidinase in brain and retina particularly to determine if brain and retina can make their own creatine, and (3) the biosynthesis of δ-guanidinovaleric acid and guanidinotaurine which are normal constituents of mammalian urine.

Part B included: No

Serial No. NHI-233
1. Lab. of Clinical Biochemistry
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Biosynthesis and Metabolism of γ-Guanidinobutyric Acid and γ-Aminobutyric Acid. II. Homopantothenic Acid

Principal Investigator: John J. Pisano

Other Investigator: Jean D. Wilson

Man Years:
Total: 0.83
Professional: 0.83
Other:

Patient Days:

Project Description:

Homopantothenic acid is pantoyl-γ-aminobutyric acid. This compound is reputed to occur widespread in the plant kingdom and in mammalian tissues, notably brain. Its function is unknown.

The first approach to this problem was to determine if brain CoA contains some γ-aminobutyric acid in place of β-alanine. A simple technique was developed for the concentration of CoA from brain. The concentrate is presently being chromatographed to determine if it contains γ-aminobutyric acid.

Homopantothenic acid was synthesized and studied for its vitamin activity. It cannot replace pantothenic acid in the diet of the rat or in Lactobacillus arabinosus. It inhibits the utilization of pantothenic acid in the micro-organism.

The development of a chemical assay for γ-aminobutyric acid has been undertaken.

Present research is directed towards (1) the determination of the amino acid content of brain CoA, (2) the vitamin or antagonistic properties of homopantothenic acid in a variety of species, (3) the quantitation of the anti-metabolite behavior of homopantothenic acid in L-arabinosus, (4) the determination of homopantothenic acid activity in enzymatic systems requiring pantothenic acid, (5) the biosynthesis of homopantothenic acid, and (6) the chemical assayfor γ-aminobutyric acid.

Part B included: No

Form No. ORP-2
Oct. 1957

PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
Laboratory of Kidney and Electrolyte Metabolism

Estimated Obligations for FY 1959

Total:	$460,408
Direct:	$320,000
Reimbursements:	$140,408

Serial No. NHI-234
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

PART A.

Project Title: Effect of Strophanthidin, adrenal steroids, and Vasopressin on water and electrolyte excretion in the chicken.

Principal Investigator: Jack Orloff

Other Investigator: Maurice Burg

Man Years:
Total: 2/3
Professional: 2/3
Other: 0

Patient Days: None

Project Description -

Progress:

The chicken was chosen for these studies for reasons outlined in detail in the last progress report. The renal-portal circulation makes it possible to investigate direct tubular effects of various agents by injecting the test substance into a leg vein and comparing urine obtained separately from each kidney Changes in composition of urine from the injected side are always due to tubular effects.

In the previous report it had been noted that the cardiac aglycone, strophanthidin inhibited the transport system in tubule cells by which sodium is reabsorbed in exchange for potassium and hydrogen ions. Injection of the drug uniformly resulted in an increase in water and sodium excretion and a fall in urine hydrogen ion concentration. Changes in potassium excretion varied with the experimental conditions. Strophanthidin decreased potassium excretion if initially high and enhanced or did not affect it if initially low. The results are consistent with the hypothesis regarding the site of action of the drug since the slight increase in potassium excretion observed when excretion was low initially is presumably due to the delivery of more sodium to the exchange site and resultant increased exchange despite an overall reduction in the capacity of the system.

Potassium is known to interfere with the action of strophanthidin on electrolyte transport in red cells. The interpretation of this effect is unclear. However, similar results have been noted in the chicken in that strophanthidin induced natriuresis is diminished if large amounts of potassium are injected simultaneously.

Part B included: Yes.

More recently the inhibitory effect of strophanthidin on para amino-hippurate accumulation of rabbit kidney slices has been studied. This will be reported separately. However it is of interest in the view of this interference by the drug that the excretion of PAH by the experimental kidney is markedly diminished if strophanthidin is injected into the homo-lateral leg vein. That this is not necessarily due to a decrease in blood flow to the organ is supported by the observations of a direct effect of the drug on the PAH transport system in intact kidney slices.

In association with Dr. S. Hajdu the excretion of strophanthidin was investigated. The drug is secreted by the tubule cells since the amount of active drug excreted exceeds that filtered at the glomerulus. However only 20% or thereabouts of the administered dose was recovered in the urine, more being excreted on the injected side. The presumption is that the drug may accumulate in tissues or be excreted in part in an inactive form. The latter would not be estimated using the bioassay system of Hajdu. Equal concentrations of the active aglycone were found in urine obtained from the two kidneys during studies in which electrolyte excretion was affected on one side only. This may be considered presumptive evidence that the drug acts on the contraluminal border of the tubule cell and need not be filtered to exert its action. It does not rule out the possibility, however, that it acts on the luminal border as well.

That adrenal steroids with structural similarity to cardiac glycosides may either antagonize or complement the action of the cardiac active compounds has been suggested in the past. No effect of this nature was observed with either aldosterone, 9-α fluoro-2-methyl hydrocortisone, or desoxycorticosterone in the intact chicken. Nor was any independent effect of the steroids on electrolyte excretion observed. Studies on electrolyte transport in kidney slices designed to test the hypothesis that steroids and digitalis-like compounds antagonize one another are reported separately.

The effect of antidiuretic hormone on urine osmolality has been reinvestigated in the chicken. Although it had been known that vasopressin decreases urine flow in this species, no evidence that it promoted the excretion of a hypertonic urine was available. That hypertonic urine could be produced was to be expected however since chickens possess the thin limb of Henle, an anatomical structure apparently present only in vertebrates capable of elaborating hypertonic urine. Spontaneous dehydration in the chicken has been shown to result in the excretion of moderately hypertonic urine, indirect evidence that endogenous ADH is secreted in the chicken. The administration of vasopressin into one leg during water diuresis (suppression of endogenous ADH release) has also been shown to elicit a decrease in urine flow and an increase in urine osmolality. The effect though frequently bilateral is greater on the injected side. Data such as these may be interpreted as indicating that vasopressin also exerts its effect on the contraluminal border of the tubule cell and need not be filtered to do so.

Direction of current research:

Strophanthidin and aldosterone effects in renal cortical slices of rabbits (and dogs) on electrolyte transport are being examined to provide information on the precise mode of action of these compounds. See associated progress report.

Incidental findings:

The effect of KCl on urine concentration was investigated in the chicken since previous work in the dog in this laboratory indicated that dilution may be interfered with by administration of potassium salts. This was not confirmed in the chicken despite injection of KCl into the renal portal venous circulation.

Part B.

Publications:

The Mechanism of Potassium Excretion in the Chicken. Jack Orloff and Douglas Davidson. Accepted for publication. Journal of Clinical Investigation.

Serial No. NHI-235
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Effect of cardiac glycosides and adrenal steroids on metabolism of slices of renal cortex

Principal Investigator: Jack Orloff

Other Investigator: Maurice Burg

Man Years:
Total: 1/2
Professional: 1/2
Other: 0

Patient Days: None

Project Description -

Objectives:

To study the effect of cardiac glycoside and adrenal steroids on electrolyte (Na^+&K^+) metabolism, oxygen consumption, and para-amino-hippurate (PAH) uptake of kidney slices.

Progress during the past year:

Slices of rabbit renal cortex were prepared by the techniques of Mudge. The animals were exsanguinated and the excised kidneys were chilled. 0.3 to 0.5 mm slices of renal cortex were incubated at 25°C in modified Ringer's solution for one to two hours.

The addition of 10^{-5} gm/ml of the cardiac aglycone, strophanthidin resulted in a lowering of slice potassium content and a proportional increase in slice sodium content. PAH uptake at one and two hours was diminished. Oxygen consumption was unaffected.

The reciprocal changes in cation content are presumably due to interference with a transport mechanism which takes up K^+ in exchange for extruded Na^+. Studies reported separately confirm that the influx of K^+ is specifically affected.

The decrease in PAH accumulation is either due to a direct effect of the drug on the transport (PAH) system or is secondary to the associated loss of potassium from the tissue. At low slice potassium concentrations less PAH is taken up, even in the absence of the drug. In experiments in which slice potassium was reduced by incubation in low potassium medium

Part B included: No

there was a reduction of PAH uptake similar to that seen when the same decrease in slice potassium content occurred with strophanthidin. Similarly, when the fall in slice potassium following strophanthidin was prevented by raising the level of medium potassium, the fall in PAH uptake was also prevented.

At medium potassium concentrations as low as 1-2 mEq/L, the slices normally maintain a potassium concentration of about 130-150 mEq/L of cellwater. At higher medium potassium concentrations the concentration gradient is maintained so that at 60 mEq/L in the medium, for instance, there is about 170-190 mEq/L of potassium in cell water. This probably indicates that a mechanism for active potassium uptake becomes saturated at physiological levels of extracellular potassium concentration and that rises in cell potassium concentration at higher extracellular levels of potassium are due to passive diffusion into the cells. 10^{-5} gm/ml of strophanthidin causes a marked depression of potassium transport at low levels of medium potassium. With medium potassium of 1-2 mEq/L, slice potassium is reduced from the normal 130-150 mEq/L cell water to levels of 50-60 mEq/L of cell water; at high levels of medium potassium, however, the same dose of strophanthidin causes virtually no reduction in slice potassium content. This indicates that the effect of the drug on active potassium transport is reduced by adding more potassium to the medium. On the basis of similar data in the red cell Glynn has postulated that the drug acts by competitively inhibiting the active portion of potassium influx.

It has been proposed that adrenal steroids and cardiac glycosides may have antagonistic effects on electrolyte transport. Desoxycorticosterone, 9Xfluoro 2 methyl hydrocortisone, and aldosterone were tested both independently and in combination with strophanthidin. No effect of the steroids was apparent on either slice content of Na^+ and K^+ or PAH uptake.

Direction of Current Research:

The studies with adrenal steroids are being repeated in slices from adrenalectomized dogs to establish whether the presence of endogenous steroid masks the effects of the added drugs.

Serial No. NHI-236
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Kinetics of Sodium and Potassium exchange in rabbit kidney slices

Principal Investigator: Jack Orloff

Other Investigator: Maurice Burg

Man Years:
Total: 7/12
Professional: 7/12
Other: 0

Patient Days: None

Project Description -

Objectives:

To develop a method for accurate determination of sodium and potassium fluxes in kidney slices and apply this to a study of factors known to affect renal electrolyte transport.

Progress during the past year:

The kinetics of Na^+ and K^+ transport in kidney slices has been examined in the past by comparing the uptake or loss of the specific radioactive isotope, using a different set of slices for each of the conditions studied. Such experiments lack precision since there may be large differences between individual slices and several analyses are required for a single determination, multiplying possible variation due to technical error. Also, interpretation is complicated by the fact that the isotope concentration of the medium is constantly changing.

In the method used in the present studies a single slice of rabbit renal cortex is perfused with a medium of constant isotope concentration. The slice is kept in a fixed volume of this medium within a well scintillation counter. Since the volume and isotope content of the medium within the well are constant, changes in counting rate are due to uptake or loss of isotope from the slice. The same slice can be used for both control and experimental determinations.

Preliminary studies showed that slices perfused in this manner with well oxygenated Krebs-Ringer bicarbonate solution at 25°C maintain for at least 2 to 3 hours a sodium and potassium content similar to that of freshly excised tissue.

To determine potassium efflux, a slice first loaded with K^{42} is counted during perfusion with an isotope-free medium. The resulting decrease in radioactivity may be described by a curve consisting of the sum of two exponential terms. The first component has a halftime of about one minute and is related to the rapid washout of isotope from the medium in the well and from the extracellular spaces. The second exponential has a half time of about 30 minutes and is presumably related to the efflux of isotope from the cells. The uptake of isotope by the slices from a medium containing K^{42} may be similarly analyzed.

Rate constants for both influx and efflux are determined from the level of tissue radioactivity and the rate at which it changes. These rate constants remain stable over a period of 2-3 hours in a single slice. Thus, when an initial value for the rate constants in a slice has been determined an experimental variable may be introduced and the changes in unidirectional flux due to this variable can be determined.

When strophanthidin (10^{-5} gm/ml) was added to the medium there was a decrease in K^{42} influx of about 25% without a change in efflux. This is consistent with findings in the red cell which indicate that the drug mainly affects the "active" component of potassium influx.

In other experiments the medium concentration of potassium ($K^{39}+K^{42}$) was varied, while the concentration of isotope (K^{42}) was kept constant. The rate constant for potassium efflux decreased, as medium potassium was lowered. This is consistent with the presence of "exchange diffusion" accounting for a portion of potassium efflux. However, proof of this explanation will require analysis of the effect of diffusion lag in the extracellular spaces on changes in efflux from the slice as a whole during the unsteady state. Potassium influx increased with increasing medium potassium level. Analysis of these preliminary results seems to indicate that there is an "active" portion of potassium uptake which becomes saturated at a medium potassium level below 5mEq/L. and that at higher levels the increase in influx is due to simple diffusion. This is consistent with the known relationship between slice potassium content and medium potassium concentration.

Studies of sodium kinetics have been largely unsatisfactory. Isotopic sodium influx into cells cannot be measured by the present techniques since a major portion of the isotope remains in extracellular spaces obscuring changes in cell radioactivity. The efflux of Na^{24} from kidney slices was measured by methods similar to those used for K^{42}. However, the results could not be described in terms of simple exponentials, and a variety of agents which might be expected to decrease sodium efflux had no effect. This suggested that either the cells are not initially labelled with the isotope or that they lose it too rapidly for measurement, and as a result, the measured washout is mostly from the extracellular spaces.

Direction of Current Research:

1) The influence of other agents on K+ flux is being studied. 2) The relationship between K+ flux and K+ concentration is being worked out in detail in order to determine the contribution of diffusion lag to the observations. 3) Further attempts are being made to measure trans-cellular flux of sodium.

Serial No. NHI-237

1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Regulation of Ammonia Excretion in the Dog and Rat

Principal Investigator: Jack Orloff

Other Investigator: Floyd Rector

Man Years:
Total: 1/2
Professional: 1/2
Other: 0

Patient Days: None

Project Description -

Progress:

The regulation of ammonia excretion has been studied in both dog and rat. In both, systemic acidosis is associated with urinary adaptation, i.e. ammonia excretion at the same pH is increased over that observed in non-acidotic animals. In the rat this increase may be correlated with an increase in glutaminase activity of renal cortical slices. Because of differences in the pattern of ammonia excretion in dog and rat it was suspected that enzymatic adaptation (increased glutaminase activity) did not occur in the dog.

Studies designed to test this confirmed the suspicion. Specimens of renal cortex of both acidotic and alkalotic dogs were assayed for enzyme activity. There was no increase in activity of any of the enzymes known to be involved directly or indirectly in the conversion of glutamine to ammonia in dogs in which evidence of urinary adaptation was present. Thus no significant alteration in the activity of pyruvate-activated glutaminase, phosphate-activated glutaminase, glutamic dehydrogenase or glutamic oxaloacetic transaminase was observed in either acidotic or alkalotic dogs. Nor was there any increase in the rate of hydrolysis of glutamine by intact cortical slices. This is in contrast to observations in the rat in which acidosis and urinary adaptation is associated with increases in the activity of both "glutaminases" and glutamic dehydrogenase. Furthermore intact kidney slices hydroyze glutamine more rapidly in acidotic than in normal or alkalotic rats.

Direction of Current Research:

Acute increases in ammonia excretion are observed following administration of certain aminoacids in both acidotic and normal dogs.

Part B included: Yes

The pattern of this response is being studied and compared in analogous studies in rats. Preliminary data indicate that there are marked differences in both species.

Incidental Findings: None

Part B.

Publications:

"The Effect of the Administration of Sodium Bicarbonate and Ammonium Chloride on the Excretion and Production of Ammonia. The Absence of Alterations in the Activity of Renal Ammonia-Producing Enzymes in the Dog.", Rector, F. and Orloff, J., Accepted for publication. Journal of Clinical Investigation.

Serial No. NHI-236
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Active K+ flux in reconstituted human erythrocyte ghosts and its relation to metabolism

Principal Investigator: Joseph F. Hoffman

Other Investigator: Daniel C. Tosteson

Man Years:
Total: 1/3
Professional: 1/3
Other: 0

Patient Days: None

Project Description -

General Purpose of Research:

There is a considerable body of evidence that the active transport of Na+ and K+ across the human red blood cell is coupled to its metabolism. Attempts aimed at the identification of the particular reaction (s) involved are at best indirect since the complexity of the metabolism presents many difficulties in the isolation of a single reaction. The approach of the present research has been to develop a system, using reconstituted red cell ghosts in which specific reactions can be isolated and assayed for their role in cation transport. The advantage of the ghost preparation results from the fact that known, normally, non-permeating components can be incorporated into the ghost interior, during the time of hemolysis, with subsequent restitution of its initial K+ permeability characteristics. In addition, certain physical and chemical properties of the plasma membrane have emerged which have become helpful in understanding the relationship of structure to function.

Progress during the past year:

(a) Development of an active and reproducible ghost system. It has been found that the K+ retention capacity and the K+ flux itself of reversed high K+ ghosts is temperature dependent. This is illustrated by the fact that 30 minutes incubation at 37°C increases the K+ retention 4-fold and reduces the flux some 20-fold. This recovery from hemolysis and the resetting of the membrane structure to repossess the passive cation movements characteristic of the original population applies to only about 60% of the derived ghosts. It has not been possible to reset the remaining fraction although this fraction can be diminished in size by differential density separation. Reconstituted ghosts can be stimulated to accumulate K+ against an 8-fold gradient by the addition to the medium of purine nucleosides. The K+ flux of control ghosts (no added substrate) is not affected by strophanthidin; in the presence of inosine,

Part B included: No.

strophanthidin reduces the K+ influx to the control level thus preventing K+ accumulation.

Analysis of metabolic intermediates indicate that inosine stimulates the production of ITP as well as the reestablishment of the various phosphate pools characteristic of normal intact cells. In addition lactate is produced. No differential metabolic effect of strophanthidin has been detected.

(b) Incorporation of ATP into the reconstituted ghosts. Since the magnitude of the K+ flux provides an inverse measure of the structural integrity of the plasma membrane and the initial K+ content of ghosts is also inversely related to this K+ flux, the degree of K+ retention can be used to assay the effects of compounds added to the hemolyzing mixture. Cells hemolyzed in Na_2ATP yield ghosts which have lost 90% of their original K+ concentration. Cells hemolyzed in MgATP (such that the divalent binding capacity of ATP is saturated) result in ghosts which retain approximately 70% of their original K+, and thus provide a system for the assay of ATP.

During these experiments it was found that Na_2EDTA or $CaCl_2$ behaved similarly to MATP but that hemolysis in the presence of $MgCl_2$ or MgEDTA or CaATP or CaEDTA yielded high K+ ghosts. It was further found that the agents preventing K+ retention act only at the time of hemolysis since addition after hemolysis does not result in any membrane damage; nor can the injury once produced be repaired by the addition after hemolysis of the alternate member of the neutrol complex. These experiments indicate that a divalent cation, probably Mg++, stabilize the internal molecular arrangement of the membrane and is necessary for normal monovalent cation permeability.

(c) The effect of incorporated ATP on the K+ flux of reconstituted ghosts. Ghosts containing ATP possess a 3-fold greater initial influx (over the 1st hour) than do control ghosts (no ATP). This increased flux is strophanthidin sensitive; after the first hour the flux falls to the control level and no longer shows any strophanthidin sensitivity. Addition of inosine stimulates the influx of ATP-containing ghosts to some 8-fold above the control showing that the active transport mechanism is not operating at maximum capacity even though the ghost contains considerable ATP (and ITP). Addition of a trace of inosine, sufficient to stimulate the pump for only a few minutes, accelerates the K influx to 4-fold over the first hour (compared to 3-fold without). These results are interpreted as indicating that ATP can act as a source of energy for the active transport mechanism but that ATP is only a link in the couple and that some other unidentified metabolic product is necessary for activation.

Direction of Current research: The above observations are being extended: (1) a more detailed analysis of both the flux and the metabolic state during the time-course of assay. In addition the effects of various inhibitors and possible activators are being studied. (2) the role of other incorporated intermediates is likewise being assayed.

Incidental findings of significance: None

Serial No. NHI-239
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Alkali cation movement in human erythrocytes after ultraviolet irradiation

Principal Investigators: John S. Cook (Guest Worker)
Joseph F. Hoffman

Other Investigator: None

Man Years:
Total: 1/3
Professional: 1/3
Other: 0

Patient Days: None

Project Description -

General Purpose of Research:

Red cells exposed to ultraviolet light undergo a colloid osmotic swelling which leads eventually to hemolysis. From previous work it was found that the rate of this hemolysis was proportional to the square of the total ultraviolet dose. The purpose of this work is to characterize the nature of the radiation lesion in terms of membrane permeabilities as an approach to understanding the dose-squared relationship.

Progress from June 1958 to September 1958:

The principle observations on the movements of Na and K across the irradiated red cell membrane are as follows: (1) intracellular K escapes according to first order kinetics and that (2) the rate constant for K outflux is proportional to the square of the total dose of ultraviolet light over the five-fold range tested. (3) the K influx is proportional to the concentration of K in the medium (for all doses). (4) the ratio of the inward to outward rate constants for K, at different doses, is equal to the chloride ratio i.e. 1.3. (5) For all doses tested Na influx was found to be proportional to the concentration of Na in the solution of origin and that (6) the ratio of the rate constants of Na influx to K outflux is found to be constant and equal to the ratio for passive permeability of unirradiated cells i.e. 0.8.

Conclusion:

Ultraviolet light greatly increases the flux of both K and Na across the membrane of the red blood cell. The cell behaves as though

Part B included: No.

large diffusion shunts have been produced in the membrane by the action of ultraviolet light. The extent to which these diffusion pathways are opened is proportional to the square of the radiation dose. In the dose range tested, ultraviolet radiation showed no differential effects on Na and K permeability.

Direction of current research:

(being carried out elsewhere)

Incidental findings of significance:

None

Serial No. NHI-240
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Cation transport in high and low potassium sheep red cells

Principal Investigators: Joseph F. Hoffman
Daniel C. Tosteson

Other Investigator: None

Man Years:
Total: 1/3
Professional: 1/3
Other 0

Patient Days: None

Project Description -

General Purpose of Research:

Some individual sheep have red cells with high potassium and low sodium (HK type) concentrations while other sheep have red cells with high sodium and low potassium (LK type) concentration. Recent evidence of Evans suggests that the LK character is inherited as a Mendelian dominant. The purpose of this research is to determine experimentally the Na and K permeabilities of these two types of sheep and to test quantitatively a derived formal description of these membrane properties. The successful application of this theory links together the membrane characteristics necessary for the maintenances of the differences in concentrations of Na and K in each type of red cell and provides an explanation for the mechanism of osmoregulation in individual cells.

Progress during the past year:

A detailed comparison of the K and Na transport processes in HK and LK sheep red cells has been made. Measurements designed to separate active transport, exchange diffusion and passive diffusion components of K and Na fluxes by techniques involving variations in the composition of the medium and the use of different substrates and strophanthidin permit the following conclusions: (1) an active transport component of K influx occurs in LK as well as HK cells but is four times greater in the latter type. (2) an active transport component of Na outflux has been identified in both cell types. (3) a large fraction of the total Na flux occurs by exchange diffusion in both HK and LK cells. (4) the passive permeability to Na and K of HK and LK cells differ markedly. LK cells have a greater passive permeability to K and a smaller passive permeability to Na than

Part B included: No.

do HK cells. It appears that a single gene controls both the magnitude of active transport and the resistance to passive diffusion of Na and K in sheep red cells.

Direction of current research and Incidental findings of significance:

The metabolism of HK and LK red cells is being studied under various conditions and will be reported in greater detail subsequently. The two cell types, under normal conditions, appear to consume glucose and produce lactate at comparable rates. In addition, there seems to be no difference in the labelling pattern of phosphate or in the kinds or pool sizes of the organic intermediates found in the two cell types.

Serial No. NHI-241
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Determination of the True Chloride Content of Tissues

Principal Investigator: Ernest Cotlove

Other Investigator: None

Man Years:
Total: 1
Professional: 1
Other: 0

Patient Days: None

Project Description -

General Purpose of Research:

Chloride is known to be the predominant anion present in extracellular fluid, but its concentration in cell fluid has been very uncertain. Reliable information on intracellular chloride would be important in understanding the behavior of electrolytes in the body. The difficulties in determining intracellular chloride have been two-fold: (1) the lack of a reliable criterion for evaluating the various analytic methods for total tissue chloride which have yielded widely disparate results; and (2) the lack of a reliable method for estimating extracellular volume, and thereby the fractions of total tissue chloride which are extracellular and intracellular.

Progress During the Past Year:

The first of the problems noted above has been solved by application of the principle of isotope dilution, involving complete mixing of tissue chloride with radioactive chloride-36, and chemical isolation of chloride in successive stages of purification to constant specific activity. This procedure has provided an absolute standard of reference for evaluation of simplified methods.

The stages in the isotope dilution method are: (1) a sample of fresh or dried tissue is completely digested in hog dilute alkali with added Chloride-36 providing complete mixing in solution of stable and radioactive chloride; (2) a portion of the alkali solution is dried, ashed at high temperature, and the organic-free residue redissolved; (3) a portion of the ash solution is treated with acid permanganate in a Warburg-type flask, oxidizing chloride to chlorine gas which diffuses into the center-well solution of very dilute alkaline hydrogen peroxide and is reduced to chloride (oxidation-reduction of (O-R) solution);

Part B included: No

(4) a portion of the (O-R) solution is dried and treated with concentrated sulfuric acid, liberating gaseous hydrogen chloride which diffuses into very dilute sodium hydroxide (distillation of (D) solution). Employing methods developed in this laboratory and described in earlier reports, the solutions at each step were counted (with the infinite thickness liquid counting method) and titrated (with the automatic coulometric-amperometric method), and the specific activity obtained as the ratio of counting rate to titration value. The (O-R) and (D) solutions of the last two stages contain chloride free of chemical interferences, as confirmed by the equality of specific activity, which in these stages represents thecounting rate per microequivalen of true chloride. The true chloride content of the tissue sample is then calculated as the ratio of the counting rate of the first-stage solution to the average specific activity of the (O-R) and (D) solutions.

In a variety of tissues of different species, the true chloride content was found to be near the lower limit of the range of values in the literature. The results of some of the methods previously reported by other workers are two to five times higher than true chloride content.

Several simplified non-isotopic analytic procedures have been tested: extractions of dried tissue with (1) water, or dilute solutions of (2) potassium nitrate, (3) nitric acid, or (4) alkaline (trisodium) phosphate, the extracts in each case being directly titrated, using the automatic titrator; and (5) an alkali-digest procedure (digestion with hot dilute sodium hydroxide, followed by protein precipitation with nitric acid, removal of interfering sulfhydryl groups by oxidation with perforate, and titration). Compared with the true chloride method, procedure (5) gave somewhat erratic results which tended to be low by several percent; extractions (1) through (3) gave results low by 1 to 9%, the lowest being with nitric acid extraction (commonly used in previous methods). Extraction with alkaline phosphate, (4), gave results within ± 3% of true chloride, and thus far appears to provide a very simple and reliable method for routine use.

Direction of Current Research:

The method of alkaline phosphate extraction will be further tested to assure its reliability for routine use. Attempts will be made to improve the non-isotopic alkali-digest method, since it would be useful for some applications. The first problem noted under "General Purpose of Research" has been solved successfully. The second problem will be approached by the use of C-14 labeled inulin and sucrose to measure extracellular spaces in rat tissue, which when combined with measurements of the total, true chloride of tissues will enable estimation of intracellular chloride. Thesubsidiary problem noted in the next section will be explored further.

Incidental Findings of Significance:

The finding of different degrees of chloride extraction by various solutions suggests an interesting type of chloride binding. Some of the binding may be of the usual electrostatic type, as indicated by the lowest degree of chloride extraction in nitric acid (where the protein charge is predominantly positive), but there also appears to be another type of binding, perhaps involving metal chelate bonds in protein. This is implied by the displacement of chloride by an excess of phosphate ion but not by an excess of nitrate ion, since the phosphate ion would compete best for a position in a metal chelate complex. The binding data obtained thus far have been on oven-dried tissues with some protein denaturation, and similar studies will be done on fresh tissues to evaluate chloride binding to native tissue protein.

Serial No. NHI-242
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The effect of potassium on the inotropic action of cardiac glycosides.

Principal Investigators: Edward Leonard
Stephen Hajdu

Other Investigators: None

Man Years:
Total: 1
Professional: 1
Other: 0

Patient Days: None

Project Description -

Objectives:

The administration of potassium chloride for the purpose of abolishing the clinical toxicity of cardiac glycosides is now widely practised. The present research was undertaken to determine whether increases of extracellular fluid potassium concentration caused any detectable inhibition of the positive inotropic action of the glycosides.

Progress:

Isolated heart of frog, strips from the right ventricle of guinea pig and rabbit, and papillary muscle of the cat were used in these experiments. The general protocol was to determine the normal twitch-tension frequency curve, and then to obtain the same data in the presence of strophanthidin both at normal and at elevated extracellular potassium concentrations. It was found that 50% increases in extracellular potassium concentration had no significant effect on the force-frequency curves of the digitalized tissue. It was concluded that the action of strophanthidin on the contractility of the heart muscle was not affected by potassium changes of this magnitude.

Direction of current research: Project concluded.

Incidental findings: None

Part B included: Yes.

Part B.

Publications:

Leonard, E. and Hajdu, S., The Effect of potassium on the intropic action of Cardiac glycosides. Clinical Research Proceedings, in press.

Serial No. NHI-243
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Investigation of a cardiotonic protein system found in increased amounts in the serum of patients with hypertension.

Principal Investigatom: Stephen Hajdu
Edward Leonard

Man Years:
Total: 1
Professional: 1
Other: 0

Patient Days: 95

Project Description -

General Purpose:

The investigation reviewed in the last two annual reports showed that a protein system found in the plasma of various mammalian species exerts a positive inotropic effect on the isolated frog heart. The protein system consists of three components. The concentration of one of them (comp L) determines the activity of the whole system of the frog heart, and becomes bound strongly to the frog heart. The activity of the system varies in different species, being rather low in the normal human for example, and high in dogs, cats, and in many patients with severe hypertensive disease. The general purpose of this research is to determine the physiological significance of this system. The first step, the separation of the three components needed for activity on the frog heart, was described in the last annual report. Currently an attempt is being made to determine the effect of the system on various mammalian tissues and to find out what kinds of interventions might alter the activity of the system.

Progress:

I. Effects of system on various tissues

a. Peripheral vascular system of the frog. A modified Trendelenburg preparation was used, which allowed measurement of the rate of flow of perfusion fluid through the hind limb vasculature. Dog component L, when combined with the rest of the system, caused marked vasoconstriction.

b. Smooth muscle of large arteries. Carotid artery strips of the rabbit, and aortic strips of rat, guinea pig, and bullfrog were studied. Addition of the protein system had no significant effect.

Part B included: Yes.

c. Isolated hearts. Strips of right ventricle from guinea pig and rat and papillary muscle of cat and rabbit were studied. Addition of the protein system caused detectable effects in the rat and guinea pig preparations, but not in the case of rabbit or cat.

d. Infusion experiments. The protein system was infused intravenously into rats and kitten, while continuously recording heart rate and blood pressure. In none of these preliminary experiments did any significant change in either of these parameters occur, despite the fact that a large amount of material was administered over a 2-3 hour period.

II. Effects on the system of altering blood pressure.

In three patients with essential hypertension whose sera regularly had high activity when tested on the isolated frog heart, activity decreased when blood pressure was lowered by administration of a ganglionic blocking agent. A more detailed analysis of the sera of two of these patients showed that the concentration of component L in these sera was still high, but that activity was depressed by the presence of an inhibitor. Preliminary investigations on the nature of this inhibitor are now in progress. Inhibitor has also been found occasionally in dog sera and regularly in the sera of a small series of adult cats. An attempt is now being made to determine whether the concentration of inhibitor can generally be influenced by hemodynamic changes.

III. Activity of the protein system in patients with aortic stenosis or coarctation of the aorta. Since the increased activity in patients with essential hypertension might be a result of the increased pressure against which the left ventricle is required to work, it was of interest to test sera of patients in whom the left ventricle was faced with a similarly increased load for other causes. The number of sera in this group tested has been small, and whereas some fell close to the normal range others have exhibited high activity comparable to levels found in patients with severe hypertension.

Direction of current research:

The physiological significance of this protein system for the whole organism is certainly unknown at the present time. That it has something to do with the cardiovascular system is suggested by the facts that (1) it acts on both heart and peripheral vasculature of the frog; (2) it is increased in certain patients with hypertension as well as with other conditions in which the isometric tension of the left ventricle during systole is above normal; and it has not been found to be increased in a variety of patients with other diseases; (3) it may become decreased, through the elaboration of an inhibitor, when the blood pressure is lowered. So far, however, a convincing cardiovascular effect when the system is given in large amounts to an intact mammal has not been demonstrated. Further research will be directed, therefore, toward determining what function the system has in intact animals.

Incidental findings of significance: None

Part B:

Publications:

(1) Hajdu, S. and Leonard, E. A serum protein system affecting contractility of the frog heart present in increased amounts in patients with essential hypertension. Circulation Research, in press.

(2) Hajdu, S. and Leonard, E. The Cellular Basis of Cardiac Glycoside Action. Pharmacological Reviews, in press.

Serial No. NHI-244
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A study of the concentrating and diluting processes in the mammalian kidney

Principal Investigator: John R. Jaenike

Other Investigators: George A. Bray, Jr.
R. W. Berliner

Man Years:
Total: 1
Professional: 1
Other: 0

Patient Days: None

Project Description -

Objectives:

An elaboration of the mechanisms operative in the concentration and dilution of the urine.

Progress During the Past Year: (Started work 7/1/58)

The major emphasis has been on setting up methods for the evaluation of changes occurring in the distal renal tubular system and in the interstitial fluid of the kidney in water diuresis and in antidiuresis. These have included:

(1) A modification of the conventional stop-flow analysis which largely eliminates the pelvic dead space and allows collection of small samples which directly reflect alterations in urine composition during the period of stopped flow.

(2) The use of sodium sensitive electrodes, developed by Dr. Murray Eden, to measure directly changes in sodium concentration within the renal parenchyma during induced changes in free water clearance in the dog.

(3) The development, by Dr. George Bray in this laboratory, of techniques for radio-autography in kidney sections

(4) Evaluation of a micro-method for sodium determination, which has been devised by Dr. Robert Bowman.

Part B included: No.

Direction of Current Research:

The following studies are underway at the present time:

(1) The change in osmolality and Na concentration in the distal collecting tubules during stopped flow, under conditions of (a) water diuresis, and (b) water diuresis with intravenous pitressin injection during stopped flow.

(2) The influence of passive urea diffusion from bladder urine to serum on the clearance ratios of inulin and urea at low urinary flow rates in the dog.

(3) The effect of K deficiency in the dog on (a) the interstitial Na concentration in the renal papella during dehydration, and (b) the effect of pitressin on the osmolality of distal collecting tubule urine during stopped flow. This study is directed at a clear definition of the renal concentrating defect in K deficiency.

(4) Radioautographic studies of the rat kidney following administration of I^{131} diodrast, in an attempt to localize areas of relative urinary concentration and dilution along the course of the nephron, with particular emphasis on the loop of Henle.

Incidental findings of Significance: None

Part B included: No.

Serial No. NHI-245
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies on Diuretics

Principal Investigator: T. J. Kennedy, Jr.

Other Investigator: None

Man Years: Patient Days: 279
Total: One-Third (1/3)
Professional: One-Third (1/3)
Other: None

Project Description:

Objectives:

The general idea of this type of study is to utilize the effect of diuretics to elucidate the basic mechanisms involved in the renal transport of electrolytes.

Progress during the past year:

Studies undertaken in 1957 on the diuretic effects of chlorothiazide were completed. Final conclusions were largely those anticipated in the previous years report. Chlorothiazide is an effective oral diuretic in those instances of edema in which salt tolerance is not too badly impaired and in which severe secondary hyperaldosteronism does not exist. While not as potent as mercurial diuretics, it has advantages in terms of ease of administration, etc. that make it a valuable therapeutic agent in clinical situations characterized by edema. In those more severely ill patients whose salt tolerance even at bed rest is very low and in whom severe secondary hyperaldosteronism exists, the drug is usually without significant effect on sodium excretion even in instances where mercurial diuretics alone or with ammonium chloride are effective. Often, in such patients, exhibition of chlorothiazide results in the induction of potassium depletion with occurrence of hypokalemic alkalosis.

Direction of Current Research:

Studies on chlorothiazide as a diuretic are essentially completed.

Incidental findings of significance:

Studies on one of several patients in this series who presented with hyponotremia, hyperkalemia and acidosis were also completed. The

Part B included: Yes.

basis for this syndrome was thought to stem from the combination of severe restriction in the rate of formation of glomerular filtrate and of severe secondary hyperaldosteronism. These limited the amount of sodium reaching the more distal segments of the nephron, and thereby precluded the formation of a dilute urine, the excretion of potassium and the excretion of hydrogen ions, and led to dilution of body fluids, retentions of potassium and retention of acid. The efficiency of osmotic diuretics, of NA_2SO_4, of mercurial diuretics and of water restriction were studied comparatively in the management of one of these patients.

A patient presenting as a puzzling case of hypokalemic alkalosis, without evidence of hyperaldosteronism was studied and discovered to have factitious disease initiated and perpetuated by consciously or subconsciously self induced vomiting.

Part B included: Yes.

Part B.

Publications:

1. Discussion, Part II, Chlorothiazide and other diuretic Agents. Ann. N.Y. Acad. Sci., 71, 439, 1958.

2. Hyponatremia. Circulation. (in press)

Serial No. NHI-246
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies on the Function of Single nephrons

Principal Investigator: T. J. Kennedy, Jr.

Other Investigator: None

Man Years: Patient Days: None
Total: One-third (1/3)
Professional: One-third (1/3)
Other: None

Project Description:
General Purpose of Research:

To attempt to establish quantitatively the rates at which specific processes occur in different segments of the nephron.

Progress in the past year:

A microchloride method has been set up. This method is capable of measuring from about 1 to $100X10^{-12}$ mols of chloride. The exact precision is at the moment indeterminate but probably no more than ± 7%. The major source of error is probably a pipetting error, and methods for reduction of this error are being studied.

Direction of current research:

1. To study the composition of urine from blood, interstitial fluid, loops and collecting ducts of hamster papillae.
2. To study the effects of experimental manipulation on the EMF's across the nephron of necturus.

Incidental Findings of Significance: None

Part B included: No.

Serial No. NHI-247
1. Kidney and Electrolyte Metebolis
2.
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Acidification of the Urine

Principal Investigator: T. J. Kennedy, Jr.

Other Investigators: R. W. Berliner
Murray Eden
Norman Levinsky

Man Years:
Total: 1/3
Professional: 1/3
Other: None

Patient Days: None

Project Description -

Objectives: The purpose of the present studies was to try to determine the significance of observed dispairities between the CO_2 tension of blood and urine as it pertained to the mechanism of urinary acidification.

Progress during the past year:

In view of the known kinetics of the H_2CO_3 dehydration in the presence and absence of buffers, it seemed possible that preurine containing significant amount of hydrated CO_2 might enter the collecting ducts when the buffer concentration of the urine was high. As the dehydration reaction, yielding CO_2 proceeded in this segment, the concentrating mechanism in the medulla, trapping CO_2 could raise the interstitial CO_2 tension. Intravenous administration of carbonic anhydrase might be expected to accelerate the dehydration reaction sufficiently to reduce the H_2CO_3 concentration of the preurine to the equilibrium concentration thus obviating the formation of extra CO_2 in the collecting ducts and the rise in medullary interstitial CO_2 tension. It was postulated that the measurement of the medullary pH, by the insertion of a microglass electrode into that part of the kidney, should reflect the CO_2 tension in the medullary interstitial fluid, and should furnish a criterion for the acceptance or rejection of this hypothesis.

Accordingly, experiments of the type alluded to were performed, during the course of which the CO_2 tension of urine was elevated by the intravenous infusion of buffer (phosphate); urine was collected from

Part B Included: No

ureteral catheters, and plasma CO_2 tension independently varied, as required, by control of depth and frequency of respiration (pump) and of chemical composition of inspired gas.

The microelectrode when inserted in the kidney or into skeletal muscle followed the plasma CO_2 tension rather nicely, and the qualitative variation in pH was in the direction expected, respiratory acidosis being associated with a fall in "tissue" pH and respiratory alkalosis by a rise.

The medullary pH in most experiments proved to be close to that of plasma, generally being slightly lower, but obviously difficult to interpret quantitatively. However, it did not seem to be directly correlated with the CO_2 tension of the urine, remaining relatively stable as the urine CO_2 tension varied widely. Finally, and most importantly, when a large disparity between urine and plasma CO_2 tension was abolished by intravenous administration of carbonic anhydrase, the expected sharp rise in the medullary pH was not observed.

Direction of current research:

It seems quite clear that either the theory proposed is invalid or that the instrument is inadequate to demonstrate the phenomenon. The electrode used was rather crude, had a fairly long active surface, and thus measured a pH integrated over a substantial depth of medulla (±3MM.). In addition, the area from which measurements were made included intra and extracellular fluids, blood and urine. It seems obvious that technically, there is much to be desired in terms of the discrete measurement of interstitial fluid pH. On the other hand, the almost totally negative impact of these experiments do not furnish much encouragement for eventual validation of the thesis. The project has been terminated, but may be reinvestigated later with more delicate electrodes and generally improved technique.

Incidental findings of significance: None

Part B included: No.

Serial No. NHI-248
1. Kidney & Electrolyte Metabolism
2. Experimental Cardiovascular Disease
3. Bethesda, Md.

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Physiology of Congestive Heart Failure

Principal Investigator: James O. Davis

Other Investigator: Nicholas A. Yankopoulos

Man Years:
Total: 2
Professional: 2
Other: 0

Patient Days: None

Project Description:

Objectives:

Area I. To determine the mechanism of increased aldosterone secretion in secondary hyperaldosteronism.
Area II. To define the biochemical defect in the failing myocardium.

Progress During the Year:

Area I. Mechanism of Aldosterone Secretion in Secondary Hyperaldosteronism.

Project I

1a. Title: Increased Aldosterone Secretion Following Acute Constriction of the Inferior Vena Cava.
1b. Investigators: James O. Davis, Bernard Klimm, Nicholas A. Yankopoulos and Ralph E. Peterson with the surgical assistance of Alfred Casper.
1c. Progress During the Year: Project is complete and paper has been accepted for publication in the Journal of Clinical Investigation.

The effects of acute constriction of the thoracic inferior vena cava (7 dogs) and of the abdominal inferior vena cava above the adrenals (4 dogs) on aldosterone, corticosterone and Porter-Silber steroid secretion in adrenal vein plasma were studied. Three of the dogs with thoracic caval constriction received dextran intravenously to maintain or to increase plasma volume. After 3 control determinations 30 min. apart, 6-8 measurements were made at similar intervals during the experimental period. Following thoracic caval constriction alone, aldosterone secretion increased within 30 min. and reached levels 2-4 fold greater than the average control rates of .008-.024 micrograms/min.; corticosterone and

Porter-Silber steroid output remained unchanged or decreased. Plasma volume was reduced. However, similar increases in aldosterone secretion occurred despite increased vascular volume secondary to infused dextran in 3 other animals with thoracic caval constriction. Two of 4 dogs with abdominal caval constriction showed increased aldosterone secretion; this occurred only after marked caval constriction and large sustained increments in venous pressure. The data demonstrate increased aldosterone secretion following acute constriction of the inferior vena cava above or below the hepatic veins and irrespective of changes in vascular volume.

Project II

2a. Title: Acute Effects of Hypophysectomy and Subsequent Diencephalic Lesions on Aldosterone Secretion in Dogs with Chronic Experimental Ascites.
2b. Investigators: James O. Davis, Nicholas A. Yankopoulos, Robert C. Bahn, Bernard Kliman, and Ralph E. Peterson.
2c. Progress During the Year: Project is complete and paper almost ready for press.

The effects of hypophysectomy and of diencephalic lesions on aldosterone and corticosterone secretion in adrenal vein plasma were studied in 10 dogs with chronic experimental ascites. During preliminary observations in 3 animals, diencephalic lesions were made by the oral approach but the pituitary was damaged; in 2 of the dogs aldosterone and corticosterone secretion fell markedly. In the third animal, ACTH was given following the lesion and aldosterone failed to fall while corticosterone declined slightly. In the definitive study (7 dogs), the pituitary was removed first before the diencephalic lesion was made. During control observations, aldosterone secretion was markedly elevated (.127 ug./min. compared with the average rate of .024 ug./min. for normal dogs); following hypophysectomy, a 76 to 97% decrease in aldosterone output occurred within 2 hours. The effects of the diencephalic lesions were variable. Four types of responses occurred; aldosterone output was 1) unchanged, 2) markedly increased, 3) decreased, and 4) increased during the first hour but declined thereafter. Control values for corticosterone secretion were within normal limits; a fall of 81-99% occurred after hypophysectomy. The progressive decline in corticosterone secretion continued after the diencephalic lesions. The data from these acute observations show a striking dependence of aldosterone and corticosterone secretion on intact pituitary function. The evidence on the relation of the diencephalon to aldosterone secretion was inconclusive but an intact middle and posterior hypothalamus was not essential for normal or elevated rates of aldosterone secretion.

Project III

3a. Title: Subacute and Chronic Effects of Hypothalamic Lesions on Aldosterone and Sodium Excretion in Dogs with Chronic Experimental Ascites.
3b. Investigators: James O. Davis, Robert C. Bahn and Wilmot C. Ball, Jr.
3c. Progress During the Year: Project is complete and paper almost ready for press.

The effects of hypothalamic lesions were studied in 10 dogs with chronic ascites produced by constriction of the thoracic inferior vena cava. During the first 3 postoperative days, the high rate of urinary aldosterone excretion and the marked Na retention characteristic of dogs with chronic experimental ascites were unaltered in all 10 animals. Every area of the hypothalamus was destroyed bilaterally in at least one animal. 7 of the 10 dogs survived the subacute 3 day period; 5 animals lived for 2 weeks or longer but 2 dogs died after 6 days. In the five chronic animals, aldosterone excretion in urine remained elevated above normal although in 3 animals a progressive decline occurred; sodium excretion remained low in all 7 dogs surviving the subacute period. In the 3 animals in which aldosterone output declined and in these dogs only, there was bilateral injury of the median eminence and adrenal atrophy. The data show, therefore, that the chronic hypothalamic lesions were without effect unless the median eminence was injured.

Project IV

4a. Title: Evidence that a Humoral Agent Stimulates the Adrenal Cortex to Secrete Aldosterone in Experimental Secondary Hyperaldosteronism.
4b. Investigators: Nicholas A. Yankopoulos, James O. Davis, Bernard Kliman and Ralph E. Peterson.
4c. Progress During the Year: Project is almost complete.

To test the possibility of a humoral efferent mechanism in the regulation of aldosterone secretion, cross circulation experiments were performed between dogs with thoracic inferior vena cava constriction and normal recipient animals. Dogs with thoracic caval constriction secrete large quantities of aldosterone and show almost complete Na retention. Cross circulation was established through the femoral vessels, or the isolated adrenals of a normal animal were perfused by the technique of Hilton, et al. (Amer. J. Physiol. 192:525, 1958) with blood from a hyperaldosteronemic donor. Control and recovery periods were obtained with the recipient's blood or by cross circulation of blood from a normal dog. Repeated determinations of aldosterone and corticosterone were made in adrenal vein plasma by the radioisotope derivative method (Fed. Proc. 17:255, 1958). Aldosterone secretion in the recipient increased 170% (7 dogs) during circulation of blood from dogs with secondary hyperaldosteronism and returned to the control level in 3 animals in which a recovery period was obtained. Corticosterone secretion increased slightly (10-30%) in 4 of 6 dogs. No consistent alterations in plasma sodium and potassium were detected. Venous pressure in the recipient dogs was unchanged. During cross circulation of blood from normal dogs into the isolated adrenals of normal animals, aldosterone secretion was unchanged or decreased. It is concluded that a humoral agent stimulates the adrenals to secrete aldosterone in dogs with secondary hyperaldosteronism.

Project V

5a. Title: Relationship of Adrenocortical and Anterior Pituitary Function to the Fecal Excretion of Sodium and Potassium.
5b. Investigators: James O. Davis, Wilmot C. Ball, Robert C. Bahn, and M. Jay Goodkind with the surgical assistance of Alfred Casper.

5c. Progress During the Year: Project is complete and paper accepted for publication in Am. J. Physiol.

The relationship of aldosterone excretion in urine to Na and K output in feces was studied. In dogs with thoracic inferior vena cava constriction and ascites, a high rate of urinary aldosterone output was associated with a very low fecal Na/K excretion. Following bilateral adrenalectomy and in the absence of hormone therapy, aldosterone was no longer detectable in urine and fecal electrolyte excretion returned to within normal limits. Hypophysectomy of dogs with thoracic caval constriction and ascites was followed by a decrease in urinary aldosterone and a concurrent rise toward or to normal of the fecal Na/K ratio. The data suggest that increased circulating aldosterone influences the intestinal mucosal transport of Na and K. Also, the effects of hypophysectomy on fecal electrolyte and renal Na excretion were observed in otherwise normal dogs. Fecal Na and K excretion increased following hypophysectomy in the majority of these animals; the increase in fecal Na output was progressive and more pronounced than that in fecal K excretion. Renal Na excretion was low during the early posthypophysectomy period but, subsequently, increased sufficiently for Na balance to be achieved in all but one dog.

Project VI

6a. Title: Acute and Chronic Studies of the Role of the Vagus, Carotid sinus and aortic depressor nerves in the control of aldosterone secretion during chronic ascites formation.

6b. Investigators: James O. Davis, Nicholas A. Yankopoulos, and John Holman.

6c. Progress during the Year: Studies were first conducted on the acute effect of bilateral cervical vagotomy on aldosterone secretion in dogs with chronic experimental ascites. The results were negative. Because of the conflicting reports, the role of the vagus has been evaluated chronically. Preliminary observations were made on the effects of chronic bilateral cervical vagotomy in a normal dog to determine if the experiment was feasible. This animal lived for 3 weeks. The effects of chronic bilateral cervical vagotomy were then studied in dogs with chronic caval constriction and ascites. Two of these dogs lived 4 days. Both dogs continued to retain Na and form ascites but Na retention was not as complete after vagotomy. The high rate of urinary aldosterone excretion was unaffected in one of the 2 dogs; measurements are not complete in the other animal. The possible importance of the carotid sinus as a peripheral receptor for hypersecretion of aldosterone in dogs with chronic caval constriction has been evaluated. Normal dogs were subjected to carotid sinus denervation. There was no effect on daily Na and K balances and on plasma Na and K and aldosterone excretion in urine (measured in 1 dog only) was normal. The thoracic inferior vena cava was then constricted in 2 dogs; aldosterone excretion in urine was increased 10-15 fold and Na retention became almost complete. No remarkable changes in plasma electrolytes were observed.

The possibility of the aortic depressor nerve as the afferent limb for a mechanism leading to hypersecretion of aldosterone has been evaluated in the rabbit. The rabbit has a separate aortic depressor nerve which does not course in close association with the vagus as in most animals. [illegible]

rabbits, no effects of bilateral aortic depressor nerve section on Na and K balances and plasma electrolytes were observed. In one of the animals, subsequent carotid sinus denervation was without effect. It is planned to constrict the thoracic inferior vena cava to produce ascites and extend the studies to evaluate the effects on aldosterone output if any positive results are obtained.

Because of above negative data, the effects of acute and chronic carotid sinus denervation on aldosterone secretion have been studied in 4 normal dogs. Data are complete on an acute and chronic animal; the results are negative.

Project VII

7a. Title: Secretion of Desoxycorticosterone (DOC) by the adrenal cortex in normal dogs and in dogs with chronic experimental ascites.
7b. Investigators: Nicholas A. Yankopoulos and James O. Davis.
7c. Progress during the year: The purpose of this study is to determine if the secretion DOC as well as that of aldosterone is increased in clinical states with ascites. Studies have been made on adrenal vein plasma from one normal dog and from one dog with chronic caval constriction and ascites. The concentrations in adrenal vein plasma were the same in the normal and the experimental animals but the rate of secretion was 100% higher in the dog with ascites.

Area II. The biochemical defect in the failing myocardium.

Project I

1a. Title: Chronic congestive failure in dogs with tricuspid insufficiency and pulmonic stenosis.
1b. Investigators: Nicholas A. Yankopoulos and James O. Davis.
1c. Progress during past year: This study was undertaken because of insufficient knowledge of physiologic changes which occur in this type of experimental failure. In particular, no evidence was available on the extent of Na retention throughout the clinical course and on the presence or absence of left ventricular failure in association with the right ventricular changes. The project is complete.

Chronic congestive heart failure was produced by the method of Barger (Am.J.Physiol. 169:384, 1952) except that pulmonic stenosis was achieved with a ligature in order to control the degree of stenosis. All of 9 dogs developed ascites. Ascitic fluid occurred after tricuspid insufficiency alone (1 animal) and following the slight stenosis produced by merely placing the ligature (3 dogs); 5 dogs required tightening of the ligature before ascites formed. Seven dogs were studied for 3-4 months. Ascitic fluid was present throughout the course but the volume of fluid varied greatly among the animals; paracentesis was not performed. On a constant Na intake, daily urinary Na excretion varied from very low excretion to that associated with Na balance; in some animals there were long periods of mild Na retention. The average increase in mean right atrial pressure [illegible] the appearance of ascites [illegible] was relatively slight [illegible]

unchanged or increased slightly. Left ventricular end diastolic pressure remained at the control level (measured for 4 months in 2 dogs only). The data show 1) a high incidence of successful preparations probably attributable to use of the technique of controlled progressive pulmonic stenosis, 2) a high RAP associated with the onset of ascites, 3) marked variability in the renal excretion of Na in the presence of chronic ascites, and 4) no evidence of left ventricular failure.

Project II

2a. Title: The electrolyte and water content of the myocardium and of the adrenal cortex from normal dogs, dogs with experimental heart failure and dogs with constriction of the thoracic inferior vena cava.
2b. Investigators: Nicholas A. Yankopoulos, James O. Davis, Mary Trapasso and Ernest Cotlove.
2c. Progress during past year. The purpose of this study, was to evaluate a number of factors which might alter the water and electrolyte composition of heart muscle during congestive heart failure. These factors include 1) the chronicity of the heart failure, 2) chronic passive congestion with and without heart failure, 3) hyperaldosteronemia, 4) a combination of chronic passive congestion and myocardial hypertrophy and 5) chronic passive congestive and hyperaldosteronemia. To provide situations for evaluation of these factors, the following animal preparations have been studied 1) cardiac failure produced by progressive pulmonic stenosis. 2) Congestive failure secondary to tricuspid insufficiency and pulmonic stenosis, 3) tricuspid insufficiency with and without hyperaldosteronemia and 4) thoracic inferior vena cava constriction with hyperaldosteronemia.

Most of the observations on cardiac muscle have been obtained but analysis of the data is incomplete. A statement of the conclusions awaits this analysis. Likewise, the incidental studies of the adrenal cortex are incomplete.

Project III.

3a. Title: Studies of the contractile proteins in the failing myocardium. I. Characterization of actomyosin by measurements of sedimentation velocity, viscosity and adenosine triphosphatase activity.
3b. Investigators: James O. Davis, Mary Trapasso and Nicholas A. Yankopoulos.
3c. Progress during past year: This project is complete and the paper is in the process of preparation.

Studies were conducted on 15 normal dogs, 7 dogs with cardiac failure produced by controlled progressive pulmonic stenosis, 5 animals with chronic congestive failure of several months duration secondary to tricuspid insufficiency and pulmonic stenosis and 3 dogs with chronic ascites produced by thoracic caval constriction. The latter preparation provided control material. Physiological studies were made throughout the course and at the time of sacrifice of the animals with chronic heart failure secondary to tricuspid insufficiency and pulmonic stenosis. There was no evidence of strain, hypertrophy or failure of the left ventricle but obvious right ventricular strain, hypertrophy and the right-sided congestive

failure syndrome were present. These observations are pertinent since it has been reported by others that actomyosin and myosin from the left ventricle are altered in dogs with congestive failure secondary to tricuspid insufficiency and pulmonic stenosis.

The yield of actomyosin was determined in muscle from both right and left ventricles; no difference was found in the actomyosin yield from normal and failing hearts. The actomyosin yield of normal skeletal muscle was higher than that of normal cardiac muscle.

Sedimentation velocity studies in an ultracentrifuge were conducted routinely on actomyosin from the right ventricle only. The typical sedimentation pattern for actomyosin from dogs with heart failure was the same as for normal dogs and for dogs with caval constriction. However, an abnormal pattern was present in actomyosin from 2 of 7 dogs with failure secondary to progressive pulmonic stenosis and in 2 of 4 dogs with chronic congestive failure produced by tricuspid insufficiency and pulmonic stenosis. A small portion of the sedimenting material had an S_{20W} ranging from 6-7. This finding was reproducible on actomyosin obtained by repeated extraction and ultra-centrifugation. In one dog with heart failure from which actomyosin showed this abnormal sedimentation pattern, actomyosin was prepared from the left ventricle. The same abnormal sedimentation pattern was present.

Studies were conducted to determine the significance of the abnormal sedimentation pattern. It was found that the slow sedimenting component could be obtained from normal cardiac muscle by repeated precipitation of actomyosin during its preparation. Measurements of pH of the material showed that the pH fell after a second or third precipitation. Similar adjustment of pH in one time precipitated material also produced this slow component. The data suggests that the abnormal sedimentation pattern with the slow component is an extraction artifact.

Upon addition of ATP to actomyosin, the sedimenting material formed a single boundary which moved at the rate of myosin A. The response to ATP was the same from all animals including the 4 dogs with the abnormal sedimentation pattern of the slow component.

Quantitative data on the rates of sedimentation of actomyosin and actomyosin+ATP which yielded myosin A showed no differences between normal and failing material. The S_{20W} for myosin A extropolated to a value of 6.25 for normal material and to similar values for pathological material. This value of 6.25 is the same as that reported independently first by Laki and Carroll and then by VonHippel Aosociates for skeletal muscle myosin.

No difference was detected in the viscosity of actomyosin from normal and failing muscle. The viscosity response to ATP of actomyosin was the same for normal and for failing material. Studies of the adenosine triphosphatase activity of actomyosin showed no difference between the activity of actomyosin from normal and failing hearts.

Summary and Conclusions: The only difference detected between actomyosin from normal and failing heart muscle was the occurrence of an abnormal sedimentation pattern in actomyosin from hearts of 4 dogs with cardiac failure; a slow boundary which sedimented at an S_{20W} of 6-7 was present. Investigation of the abnormal sedimentation pattern suggests that it is an extraction artifact.

Project IV.

4a. Title: Studies of the Contractile proteins in the Failing Myocardium. II. Characterization of Myosin A by studies of sedimentation velocity and viscosity with determinations of the molecular weight.

4b. Investigators: James O. Davis, William R. Carroll, Mary Trapasso and Nicholas A. Yankopoulos.

4c. Progress during past year: A highly purified Myosin A has been prepared and studies made of myosin A from cardiac muscle of normal dogs and of dogs with chronic cardiac failure secondary to tricuspid insufficiency and pulmonic stenosis. Also, viscosity studies have been made on myosin A from cardiac muscle of dogs with thoracic caval constriction. All comparisons of viscosity were made on myosin A from the entire heart of normal dogs and from the failing right ventricle unless otherwise stated.

In general, our viscosity data confirm the finding of Olson that the viscosity of normal and failing myosin is the same for concentrations above 1mg./ml. Below this concentration, the viscosity of myosin A decreased (Nsp/C vs. concentration) for normal myosin A and increased for myosin A from failing heart muscle. There are two exceptions, however, to this general description. Myosin A from one normal dog failed to show the decrease observed in material from 3 other normal animals. Secondly, Myosin A from the left ventricle of a dog with heart failure showed an increase in viscosity at low concentrations. Finally, myosin A from the right ventricle of dogs with caval constriction has also showed an increase in viscosity at low concentrations.

Extensive measurements of the sedimentation velocity constant for myosin A have failed to reveal any difference between myosin A from normal hearts, failing right ventricle, failing left ventricle and the right ventricle of dogs with thoracic caval constriction. The S_{20W} at zero concentration ranged from 6.1-6.4.

Recently, Dr. William R. Carroll has made measurements of the diffusion of two preparations of cardiac myosin A. Myosin A from normal heart muscle showed a diffusion coefficient, D_{20W} of 1.1×10^{-7} at zero concentration which is similar to the value of 1.0×10^{-7} reported by Laki and Carroll for normal skeletal muscle myosin A. Calculations of the molecular weight yielded a value of approximately 510,000. Similar measurements are now being made of myosin A from failing heart muscle.

Project V.

5a. Title: The water and electrolyte content and the contractile proteins of cardiac muscle from hypophysectomized dogs.

5b. Investigators: Nicholas A. Yankopoulos, James O. Davis, William Gay.

5c. Progress during past year: Studies have been conducted on cardiac muscle from 5 hypophysectomized dogs which were hypophysectomized at the age of 4-6 weeks. After 6-10 months the animals were sacrificed and the cardiac muscle studied.

The data showed no alteration in the contractile proteins. The water and fat content, however, was unusually high in some animals. Also, the concentration of Na per unit wet weight of muscle was increased. It is planned to analyze the available data more completely and to compare the findings withthose in cardiac failure. In both cardiac failure and panhypopituitarism the cardiac output is markedly reduced. By this comparison it is hoped that some light will be thrown on the basic defect in the failing myocardium.

Project VI.

6a. Title: Isometric tension Developed by Glycerol-extracted Muscle from the Normal and the Failing Myocardium.
6b. Investigators: Nicholas A. Yankopoulos and James O. Davis.
6c. Progress during past year: The purpose of this study is to examine the contractility of the myocardial proteins in a system isolated from membrane effects, energy-supplying systems, electrolyte effects and pH effects since the latter are controlled. Glycerol-extracted myocardial muscle bundles were prepared by the method of Szent-Gyorgi. Isometric tension was measured following addition of ATP by means of a Statham force transducer. Studies have been conducted on 10 fiber bundles from the right ventricle and 10 bundles from the left ventricle from 3 normal dogs and from 3 dogs with cardiac failure produced by progressive pulmonic stenosis. No difference was found in the contractility of fibers from failing as compared with normal muscle. More recently, muscle fibers from 2 dogs with chronic failure secondary to tricuspid insufficiency and pulmonic stenosis have been studied. In fibers from one of these dogs, less tension was developed. More data are needed to settle the question of whether the chronicity of heart failure is important in producing a change in the contractility of the myocardial proteins.

Direction of Current Research:

Area I.

It is planned to continue to study the afferent and efferent limbs of the aldosterone system. Exploratory studies will be made in an attempt to locate a peripheral receptor and to define the nature of the stimulus. Studies will be made to determine the source of the humoral agent which stimulates the zona glomerulosa to secrete aldosterone. Finally, a rather extensive program will be launched to isolate and identify chemically the aldosterone-stimulating hormone.

Area II.

It is hoped that the immediate problems under study will be completed soon. This program could be extended to include 1) verification

of the molecular weight of myosin A by another technique, the approach to sedimentation equilibrium, 2) interaction of highly purified normal actin and failing myosin and vice versa to form artificial actomyosin to determine if either the actin or myosin molecule is abnormal in the failing heart, and 3) studies of muscle proteins from human cardiac muscle.

The extent to which studies in Area II will be extended will depend upon available time and facilities and the relative importance of our findings compared with those in Area I.

Incidental findings of Significance: These findings have been presented under the individual projects.

Publications:

1. Davis, James O. and Wilmot C. Ball, Jr.: Effects of a body cast on aldosterone and sodium excretion in dogs with experimental ascites. Am. J. Physiol., 192:538, 1958.
2. Yankopoulos, Nicholas A., James O. Davis, Bernard Kliman and Ralph E. Peterson: Increased aldosterone secretion following acute constriction of thoracic and of abdominal inferior vena cava. Fed. Proc. 17:173, 1958.
3. Davis, James O., Robert C. Bohn, Nicholas A. Yankopoulos, Bernard Kliman, Ralph E. Peterson and Wilmot C. Ball, Jr.: Effects of acute and chronic hypotholomic lesions on aldosterone secretion in dogs with chronic experimental ascites. The Physiologist 1:15, 1958.
4. Yankopoulos, Nicholas A. and James O. Davis: Chronic congestive failure in dogs with tricuspid insufficiency and pulmonic stenosis. The Physiologist 1:88, 1958.

In Press:

5. Davis, James O., Wilmot C. Ball, Jr., Robert C. Bohn and M. Jay Goodkind. Relationship of anterior pituitary and adrenocortical function to the fecal excretion of sodium and potassium. Am. J. of Physiol. (Accepted for publication).
6. Davis, James O., Bernard Kliman, Nicholas A. Yankopoulos and Ralph E. Peterson. Increased aldosterone secretion following acute constriction of the inferior vena cava. J. Clin. Invest. (Accepted for publication).

Form No. ORP-2
Oct. 1957

PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
Surgery Branch

Estimated Obligations for FY 1959

Total: $1,083,568

Direct: $355,000

Reimbursements: $728,568

Serial No. NHI-249
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Left Heart Catheterization

Principal Investigator: Andrew G. Morrow, M. D.

Other Investigators: Estelle Cohen
Fred Bullock
William Laughlin

Man Years (calendar year 1958):
Total: 1
Professional: 6/12
Other: 6/12

Patient Days (calendar year 1958): 1000

Project Description:

Numerous previous reports have dealt with the technique and application of left heart catheterization in the assessment of patients with various forms of heart disease. As of this date, 900 transbronchial left heart catheterizations have been performed without a death or serious sequel. There has been increasing application of left heart catheterization in the study of patients with congenital heart disease. Patients with congenital aortic stenosis have been of particular interest and their assessment by this method has proved a valuable adjunct in management. Indicator-dilution curves with left heart injection are also being carried out with increasing frequency.

Other methods of left heart catheterization are also being used. Percutaneous puncture of the left ventricle has been carried out in nearly 100 patients. This technique is of particular applicability in young children and in those patients with severe mitral valve disease in whom the catheter cannot be passed into the left ventricle bronchoscopically. In many instances a combined transbronchial and percutaneous approach has been used.

Proposed course of project: The studies outlined above are being continued. Selective angiocardiography by means of direct puncture of the left atrium or left ventricle have been carried out in a limited number of patients. This technique showed great promise in the evaluation of mitral insufficiency, the localization of aortic stenosis, and the detection of left-to-right shunts.

Part B included Yes

Serial No. NHI-249

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B:

Article in Periodical:

Morrow, A. G., Braunwald, Eugene, and Sharp, Edward H.:
The Clinical Features and Surgical Treatment of Congenital Aortic Stenosis. Progress in Cardiovascular Diseases, Vol. 1, No. 1, pp. 80-88, 1958.

Morrow, A. G., Braunwald, Eugene, and Sharp, Edward H.:
Congenital Aortic Stenosis: Clinical and Hemodynamic Findings, Surgical Technic and Results of Operation. Circulation, Vol. 18, No. 6, pp. 1091-1104, 1958.

Serial No. NHI-250
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Development and Clinical Application of a Modified Kay-Cross Artificial Heart and Lung Machine

Principal Investigator: Andrew G. Morrow, M. D.

Other Investigators: Joseph W. Gilbert, M. D.
John A. Waldhausen, M. D.
Robert T. L. Long, M. D.
Clarence S. Weldon, M. D.
John Ross, M. D.
Estelle Cohen
Robert Carr
Fred Bullock

Man Years (calendar year 1958):
Total: 4
Professional: 2
Other: 2

Patient Days (calendar year 1958): 2500

Project Description:

A previous report has detailed experiences with the Melrose artificial heart and lung machine evaluated in this unit. The machine was abandoned for the following reasons: the occurrence of unexplained massive bleeding in 5 patients; inability of the device to oxygenate large volumes of blood adequately; dependence upon chemical sterilization; a high incidence of air embolism.

In February 1958 a Kay-Cross oxygenator was purchased. This was integrated into a pump and control unit designed and built with the cooperation of the NIH Instrument Section. This machine, in its present form, provides full oxygenation of blood at flows of 4 L./min. and greater, may be completely sterilized with heat, is not injurious to blood, and does not produce sufficient agitation to result in air embolism. The machine has been applied in 70 patients subjected to open operations for the correction of various congenital and acquired cardiovascular lesions.

Part A. (continued)

Project Title: Development and Clinical Application of a Modified Kay-Cross Artificial Heart and Lung Machine

Project Description:

Proposed course of project: The machine is being further improved by the design and installation of an automatic blood level control, provision for constant temperature control in the reservoir and oxygenator and development of disposable oxygenator plates.

Part B included No.

Serial No. NHI-251
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Diagnosis of Pulmonic and Tricuspid Valvular Regurgitation by a Dye-Dilution Technique

Principal Investigator: N. Perryman Collins, M. D.

Other Investigators: Eugene Braunwald, M. D.
Andrew G. Morrow, M. D.

Man Years (calendar year 1958):
Total: ½
Professional: ½
Other: ½

Patient Days (calendar year 1958): 50

Project Description:

Patients with suspected incompetence of the pulmonic and/or tricuspid valves have been studied by means of the injection of the indicator dye, cardiogreen, into the distal chamber while blood is sampled continuously through a densitometer from the proximal chamber. In this manner any dye which regurgitates across the valve into the proximal chamber can be detected. A double lumen catheter was constructed in such a manner so that the lumina of the separate catheters are 5 cm. apart. Eight normal valves have been studied and it has been found that no regurgitation occurs from the catheter's having been inserted through the valve. Therefore, the appearance of dye in the proximal chamber great enough to produce a definite curve is diagnostic of valvular regurgitation.

Proposed course of project: Continued use as a standard technique for the definite diagnosis of valvular regurgitation in patients suspected of having this type deformity.

Part B included No.

Serial No. NHI-252
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A Comparison of the Usefulness of the Plasma Thrombin Time and the Protamine Titration Test in the Postoperative Neutralization of Heparin

Principal Investigator: James A. McFarland, M. D.

Other Investigators: James C. Peden, M. D.
Andrew G. Morrow, M. D.

Man Years (calendar year 1958):
Total: ¼
Professional: ¼
Other: 0

Patient Days (calendar year 1958): 30

Project Description:

A reasonably accurate and simple test is of considerable value in assessing the adequacy of heparin neutralization by intravenous protamine sulfate in postoperative patients in whom extracorporeal circulation has been employed. A simplified protamine titration test, which has been most frequently used, has proved to be cumbersome and to afford only a gross estimate of the neutralization end-point. The plasma thrombin time is known to be sensitive to very small amounts of heparin. Accordingly, heparin neutralization in 25 consecutive patients was followed using both the above tests. The thrombin time was found frequently to demonstrate the presence of circulating heparin after the protamine titration test had returned to normal and was also considered simple and less time consuming to perform.

Part B included No.

Serial No. NHI-253
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Use of Inert Gases in the Detection of Left-to-Right Circulatory Shunts

Principal Investigator: Andrew G. Morrow, M. D.

Other Investigators: Eugene Braunwald, M. D.
Richard J. Sanders, M. D.
Estelle R. Cohen
Fred Bullock

Man Years (calendar year 1958):
Total: 5-2/12
Professional: 3
Other: 2-2/12

Patient Days (calendar year 1958): 2600

Project Description:

Previous reports have outlined the usefulness of the inert gas nitrous oxide in the localization of left-to-right ciruclatory shunts and the advantages of this method over the determination of oxygen differences has been proved. In the past year the inert gas Krypton^{85} has also been employed in this manner. The use of a radioactive gas has the advantage that analysis of the blood samples can be accomplished very rapidly by simple counting and the results of the study are immediately available. Kr^{85} tests were carried out in 150 patients. The results were the same as those of the nitrous oxide test and the advantages over the oxygen method were similar.

Radiation safety considerations constitute a disadvantage of the radioactive gas but further refinement in technique will obviate this drawback.

Proposed course of project: The Kr^{85} tests will be continued in patients and further studies made of factors increasing its safety.

Part B included Yes

Serial No. NHI-253

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B:

Article in Periodical:

Sanders, R. J.: The Use of a Radioactive Gas (Kr^{85}) in Diagnosis of Cardiac Shunts. Proc. Soc. Exper. Bio. & Med., Vol. 97, 1-4, 1958.

Sanders, R. J., and Morrow, A. G.: The Localization of Circulatory Shunts with Inhaled $Krypton^{85}$. Bulletin of the Johns Hopkins Hospital, Vol. 103, No. 1, pp. 27-31, July 1958.

Serial No. NHI-254
1. Clinic of Surgery
2. Section on Cardiology
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A Study of Factors Influencing Digitalis Effect on the Refractory Period of the Atrio-Ventricular Node

Principal Investigator: Robert L. Frye, M. D.

Other Investigators: Eugene Braunwald, M. D.

Man Years (calendar year 1958):
Total: 4/12
Professional: 2/12
Other: 2/12

Patient Days (calendar year 1958): 220

Project Description:

Three patients with chronic atrial fibrillation have been studied to date, after discontinuation of all previous digitalis. After vagal blockade by atropine, acetyl-strophanthidin was administered at a constant rate. The acetyl-strophanthidin requirement for slowing the ventricular rate to 60 was determined. After control data had been obtained, the influence of the infusion of potassium, calcium, and nor-epinephrine on the acetyl-strophanthidin requirement was determined. Following the infusion of potassium, a slight increase in digitalis requirement was noted. It was noted that the calcium gluconate solution alone significantly decreased the ventricular rate. Further administration of acetyl-strophanthidin did not result in any noticeable synergism or toxicity. One patient has been studied during a constant infusion of nor-epinephrine sufficient to produce a 20-30 mm.Hg increase in blood pressure, but no significant difference from control data could be detected.

Proposed course of project: Additional patients are to be studied using the constant infusion of acetyl-strophanthidin. In addition, it is planned to study the effects of fever and thyroid hormone on digitalis requirement. A patient with therapeutic myxedema is being studied at the present time. Determinations in a hypothyroid state have been completed and similar studies in a euthyroid and hyperthyroid state are planned.

Part B included No

Serial No. NHI-255
1. Clinic of Surgery
2. Section on Cardiology
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Response of Cardiac Output to Infusion in a Control State and Following Ganglionic Blockade

Principal Investigator: Robert L. Frye, M. D.

Other Investigators: Eugene Braunwald, M. D.
Mrs. Estelle Cohen

Man Years (calendar year 1958):
Total: 10/12
Professional: 6/12
Other: 4/12

Patient Days (calendar year 1958): 300

Project Description:

The applicability of Starling's law of the heart to the intact human has not been proven. Studies in humans utilizing infusion of saline, albumin, and other materials have shown an inconsistent response of cardiac output to such infusion. The reason for the inconsistent response has never been explained. For this reason the present study was undertaken to determine the influence of ganglionic blocking agents on this response.

Three patients have been studied to date following the rapid infusion of 1500 ml. of the patient's own whole blood. Measurement of cardiac output before and after infusion in a control state and following ganglionic blockade with Arfonad has been made. All patients have exhibited a much greater rise in stroke volume and stroke work following infusion under the influence of ganglionic blockade. Two have shown a significant difference between the control and ganglionic blockade state as regards cardiac output.

Proposed course of project: Additional patients are to be studied in a similar manner.

Part B included No

Serial No. NHI-256
1. Clinic of Surgery
2. Section on Cardiology
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: An Analysis of Adequacy of Maintenance Doses of Digitoxin Following Rapid Digitalization with Ouabain

Principal Investigator: Robert L. Frye, M. D.

Other Investigators: Eugene Braunwald, M. D.

Man Years (calendar year 1958):
Total: 6/12
Professional: 4/12
Other: 2/12

Patient Days (calendar year 1958): 100

Project Description:

A great deal of controversy has centered about the problem of what constitutes an adequate maintenance dose following full digitalization with a rapid acting preparation such as Ouabain. Two patients with atrial fibrillation have been studied to date following rapid digitalization with Ouabain. Both patients were then started on 0.15 mgm. of digitoxin every day, 12 to 24 hours after receiving Ouabain. The amount of acetyl-strophanthidin necessary to slow the ventricular rate to a level equal to that achieved with maximum effect of the Ouabain was determined. One patient required no acetyl-strophanthidin at any time. The other patient required several injections each day. This indicated that he had lost his initial status of complete digitalization.

Proposed course of project: Additional patients are to be studied utilizing various regimens for administering maintenance doses of digitoxin. It is planned to study patients who have received 0.15 mgm. of digitoxin immediately following the peak effects of Ouabain and also patients who receive one-half of a digitalizing dose of digitoxin 12 to 24 hours following the Ouabain.

Part B included No

Serial No. NHI-257

1. Clinic of Surgery
2. Section on Cardiology
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A Study of Variations in the Pulmonary and Systemic Artery Pressures During the Respiratory Cycle

Principal Investigator: Eugene R. Kelly, M. D.

Other Investigators: Eugene Braunwald, M. D.

Man Years (calendar year 1958):

Total:	4/12
Professional:	2/12
Other:	2/12

Patient Days (calendar year 1958): 200

Project Description:

The effect of respiration on systemic and pulmonary artery pressures has been previously described in cardiovascular normal subjects. This investigation is being carried out in an attempt to ascertain whether this normal pattern is altered by the presence of heart disease. An abnormal response was noted in 10 of 16 patients. In general, patients with pulmonary hypertension or increased pulmonary blood flow tended to show an elevation of pulmonary artery pressure during inspiration, instead of the decline which is normally observed. It is likely that in these patients the increase in pulmonary artery pressure is related either to diminished distensibility of the pulmonary vascular bed or that the patient is operating on a steep portion of the pressure-volume curve of the pulmonary vascular bed.

Proposed course of project: Additional patients are to be studied.

Part B included No

Serial No. NHI-258
1. Clinic of Surgery
2. Section on Cardiology
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Hemodynamic Effects of Rapid Digitalization in Cardiovascular Normal Subjects

Principal Investigator: Eugene Kelly, M. D.

Other Investigators: Eugene Braunwald, M. D.
Mrs. Estelle R. Cohen
Mr. Fred Bullock

Man Years (calendar year 1958):
Total: 3/12
Professional: 2/12
Other: 1/12

Patient Days (calendar year 1958): 100

Project Description:

Relatively little reliable data has been available on the effects of digitalis on cardiovascular dynamics in subjects without congestive heart failure. Accordingly, cardiac output and central blood volume were measured by the indicator-dilution technic, together with arterial and central venous pressures before and 45 minutes after the intravenous administration of 0.50 to 0.75 mg. Ouabain. Left ventricular stroke work and cardiac output declined significantly in two of seven subjects, and showed no significant change in the remainder. In two subjects, central blood volume decreased significantly.

Proposed course of project: Additional subjects are to be studied. From the data it appears as if those subjects receiving only 0.50 mg. Ouabain had no significant hemodynamic changes, while those receiving 0.75 mg. had more substantial effects. It is planned to study several other subjects using 0.75 mg. Ouabain.

Part B included No

Serial No. NHI-259
1. Clinic of Surgery
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Hepatic Blood Flow During Cardiopulmonary Bypass

Principal Investigator: John A. Waldhausen, M. D.

Other Investigators: Carlos R. Lombardo, M. D.
James A. McFarland, M. D.
William P. Cornell, M. D.
Raymond Waters
Robert White

Man Years (calendar year 1958):
Total: 1-4/12
Professional: 8/12
Other: 8/12

Patient Days (calendar year 1958): None

Project Description:

There is clinical evidence that there is some liver damage during extracorporeal circulation. It was therefore felt warranted to investigate hepatic blood flow during cardiopulmonary bypass.

The latter was established using a nonocclusive roller pump and a rotating-disc oxygenator. Venous outflow was carried out by cannulation of the superior vena cava in the usual way. The inferior vena cava was drained by cannulation of the vessel just above the renal veins but below the hepatic vessels. The segment of cava into which the hepatic veins drained was cannulated separately. Hepatic venous blood was drained into a calibrated glass cylinder and measured over a given time period.

In order to study hepatic blood flow at normal cardiac output venous blood (caval blood and hepatic blood) could be returned through a flowmeter directly to the pulmonary artery at a flow of 2.8 L./M.^{2}/ min. Hepatic blood flow here constituted control. This was compared to that flow recorded on bypass. The total flow on cardiopulmonary bypass was varied. Twenty minute recordings were made at flows of 2.8 L./M.^{2}/min; 2.2 L./M.^{2}/min.; 1.8 L./M.^{2}/min.; 1.4 L./M.^{2}/min.; and 1.0 L./M.^{2}/min.

Serial No. NHI-259

Part A. (continued)

Project Title: Hepatic Blood Flow During Cardiopulmonary Bypass

Project Description:

Eight dogs in all were studied. Hepatic blood flow does not differ on cardiopulmonary bypass if the total flow is maintained at the control level. Reduction of the systemic flow to 2.2 L./M.^{2}/min. and 1.8 L./M.^{2}/min. does not reduce hepatic blood flow significantly. Flows lower than that reduce the liver flow. However, the liver flow expressed as percent of the total flow increases.

Proposed course of project: In addition to hepatic venous flow hepatic artery flow will be measured. Oxygen consumption will also be studied.

Part B included No.

Serial No. NHI-260
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Hemodynamic and Pharmacologic Observations in Experimental Mitral Insufficiency

Principal Investigator: John Ross, Jr., M. D.

Other Investigators: Theodore Cooper, M. D.
Carlos R. Lombardo, M. D.

Man Years (calendar year 1958):
Total: 9/12
Professional: 4/12
Other: 5/12

Patient Days (calendar year 1958):
NONE

Project Description:

This investigation was designed to clarify the quantitative pressure flow relationships occurring during the cardiac cycle in experimental mitral insufficiency. Clinical studies had aroused interest in a more exact definition of the quantitative relationship of regurgitation to V-wave changes in the left atrial pulse contour. In addition, several patients who were subsequently shown to have mitral insufficiency failed to demonstrate an increase in the left atrial V-wave during norepinephrine infusion, and further experimental evaluation of this technique was planned.

In six dogs, mitral insufficiency was produced by apico-atrial shunt, as described by Braunwald et al. Regurgitant flow in this loop and total forward output were measured simultaneously with electromagnetic flowmeters. The regurgitant loop was rigid, allowing undamped pressure transmission into the atrium. Little change in mean pressure and forward flow occurred; however, significant alterations in the left atrial pulse contour were present with regurgitant flows averaging twice forward cardiac output. With smaller amounts of regurgitation, the only change was an early onset of the V-wave. Maximum regurgitation occurred an average of 0.14 seconds before the V-wave peak and continued well into the relaxation phase.

Vasoxyl and Levophed were administered to two of these dogs and to an additional 10 animals, three of which were normal, the remaining seven having chronic mitral insufficiency. Levophed produced an initial tachycardia, lowering of mean left atrial

Serial No. NHI-260

Part A. (continued)

Project Title: Hemodynamic and Pharmacologic Observations in Experimental Mitral Insufficiency

Project Description:

pressure and decrease in V-wave height in all animals. Systemic output increased, regurgitation diminished. If the infusion was continued for several minutes, bradycardia supervened and V-wave height increased. This effect was magnified by prostigmine. Vasoxyl produced an immediate increase in V-wave height.

Proposed course of project: Further studies in the detailed analysis of the timing of regurgitation with left atrial pressure pulse contour are planned.

Part B included Yes

Serial No. NHI-260

Part A. (continued)

Project Title: Hemodynamic and Pharmacologic Observations in Experimental Mitral Insufficiency

Project Description:

pressure and decrease in V-wave height in all animals. Systemic output increased, regurgitation diminished. If the infusion was continued for several minutes, bradycardia supervened and V-wave height increased. This effect was magnified by prostigmine. Vasoxyl produced an immediate increase in V-wave height.

Proposed course of project: Further studies in the detailed analysis of the timing of regurgitation with left atrial pressure pulse contour are planned.

Part B included Yes

Serial No. NHI-260

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B.

Article in Periodical:

Ross, J., Braunwald, E., Morrow, A. G.: Clinical and Hemodynamic Observations in Pure Mitral Insufficiency. Am. J. Cardiol., Vol. 1, No. 1, pp. 11-23, July, 1958.

Serial No. NHI-261
1. Clinic of Surgery
2. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: An Experimental Investigation of the Use of Indicator-Dilution Curves in the Study of Portacaval Shunts

Principal Investigator: Robert T. L. Long, M. D.

Other Investigators: Carlos R. Lombardo, M. D.
Eugene Braunwald, M. D.
Andrew G. Morrow, M. D.
Henry Felton
Russell Holland

Man Years (calendar year 1958):
Total: 7/12
Professional: 4/12
Other: 3/12

Patient Days (calendar year 1958):
None

Project Description:

The use of splenic pulp or mesenteric vein injections of indicators in detecting the patency of portacaval shunts has been studied in nine dogs with side-to-side portacaval shunts. Tricarbocyanine dye curves have been recorded from the right atrium and femoral artery, and Krypton^{85} curves have been recorded from the expired air. Injection of a mixed solution of the indicators was made into the splenic pulp, a mesenteric vein, or directly into the portal vein. These curves were done with the shunts open and closed. After an injection with the shunt open, the anastomosis was clamped and injection repeated. These dogs thus served as controls when the shunts were clamped. A significant difference in appearance time, peak time, and height of the curves was present between the open and closed shunt curves. Further differentiation was made possible by comparing these curves to those obtained following peripheral intravenous injection. The intravenous curves correspond closely to those obtained with the shunt open. Patent portacaval shunts in the normal dog may be easily detected by this technique.

Proposed course of project: These studies are being initiated in patients with portal hypertension undergoing shunt surgery, as well as patients with normal portal systems undergoing laparotomy. The presence of naturally occurring portacaval anastomoses in patients with portal hypertension may make this test of value in the diagnosis of esophageal varices.

Part B included No.

Serial No. NHI-262
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Elective Cardiac Arrest Studied by Means of Ventricular Function Curves

Principal Investigator: John A. Waldhausen, M. D.

Other Investigators: Nina S. Braunwald, M. D.
William P. Cornell, M. D.
Robert Bloodwell, M. D.
Andrew G. Morrow, M. D.
Raymond Waters
Robert White

Man Years (calendar year 1958):
Total: 1-6/12
Professional: 9/12
Other: 9/12

Patient Days (calendar year 1958):
None

Project Description:

Elective cardiac arrest is well established clinically. However, it was felt that the procedure warranted an investigation of the effects of the two most commonly used arresting solutions, potassium citrate and acetylcholine on myocardial contractility. An established method of measuring the latter is by means of ventricular function curves.

The preparation here was modified from that originally described by Sarnoff et al in that total venous return to the heart except coronary blood flow was drained into a reservoir and then pumped through a Richardson Flowmeter into the pulmonary artery. Pressures were recorded in the left atrium and aorta. Thus, cardiac output, left ventricular filling pressure, and aortic pressure were known. By varying pump output and thus varying cardiac output stroke work could be correlated with various left ventricular filling pressures.

Function curves were obtained both before and after arrest. The arrest was induced while the animal was on cardiopulmonary bypass. Lengths of time of arrest were varied.

Serial No. NHI-262

Part A (continued)

Project Title: Elective Cardiac Arrest Studied by Means of Ventricular Function Curves

Project Description:

Eighteen studies of elective cardiac arrest have been done. Periods of arrest have varied from 10-30 minutes. No significant difference has been noted in regard to the choice of acetylcholine and potassium citrate on the post arrest myocardial contractility and both agents caused severe depression of function with arrest periods of 20 and 30 minutes.

Proposed course of project: The plan is to add to the study an investigation of myocardial contractility following anoxic arrest and ventricular fibrillation.

Part B included No.

Serial No. NHI-263
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: An Experimental Investigation of the Use of Radioactive Gas Solutions in the Diagnosis of Right-to-Left Cardiac Shunts

Principal Investigator: Robert T. L. Long, M. D.

Other Investigators: John A. Waldhausen, M. D.
William P. Cornell, M. D.
Henry Felton
Leander Brown

Man Years (calendar year 1958):
Total: 8/12
Professional: 4/12
Other: 4/12

Patient Days (calendar year 1958):
None

Project Description:

Eleven dogs with artifically constructed right-to-left shunts, as well as two normal dogs, have been studied experimentally. Six of these animals were studied from six to ten days postoperatively, and five were studied acutely, immediately after construction of the shunt. Patency of the shunt was proved by sacrificing the animal immediately after study and examining the anastomosis. Saline solutions of Krypton^{85} were injected into the chambers of the right side of the heart as well as the pulmonary artery. Immediately after injection arterial blood samples were drawn at twenty second intervals for 1.5 minutes. These samples were counted as whole blood using a continuous gas flow Geiger-Mueller tube. In two animals, the radioactivity of the expired air was sampled by means of a Geiger tube inserted into the airway. A marked difference in arterial blood gas content was present between dogs with shunts and control dogs. This was most marked in the 0-20 second blood sample. The maximum count in controls was 206 counts per minute for this sample. In dogs with shunts the counts ranged from 1400 to 14,000 per minute.

In normal dogs virtually all of the injected Krypton^{85} is excreted by the lungs in its first transit through the pulmonary circulation, and virtually none of it enters the

Serial No. NHI-263

Part A. (continued)

Project Title: An Experimental Investigation of the Use of Radioactive Gas Solutions in the Diagnosis of Right-to-Left Cardiac Shunts

Project Description:

arterial circulation. In animals with right-to-left shunts, however, the gas bypasses the lungs and enters the left side of the heart. Appearance time is not critical, since the diagnosis is made on the absolute level of Krypton^{85} in arterial blood.

Proposed course of project: Preparations are being made to apply this method of detecting shunts to clinical use in the cardiac catheterization laboratory.

Part B included No.

Serial No. NHI-264
1. Clinic of Surgery
3. Bethesda

PHS--NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: An Experimental Evaluation of the Use of a Decompression Chamber in the Treatment of Cerebral Air Embolism

Principal Investigator: William P. Cornell, M. D.

Other Investigators: Andrew G. Morrow, M. D.
Henry Felton
Raymond Waters

Man years (calendar year 1958):
Total: 4/12
Professional: 2/12
Other: 2/12

Patient Days (calendar year 1958):
None

Project Description:

Under general anesthesia and local anesthesia, the carotid arteries of dogs were injected cephalad with varying amounts of air. After a series of controls were obtained another group of dogs was injected with air and then placed in a compression chamber under a pressure of 4 atmospheres and slowly decompressed.

There has been no difference in the mortality in the control dogs and in the treated dogs. The amount of air a dog can tolerate varies considerably and it is thought that an animal must be used that has a more uniform response to a given amount of air.

Proposed course of project: The use of a different more thoroughbred animal is being investigated and injection of dogs through a different artery such as the vertebral is being investigated.

Part B included No

Serial No. NHI-265
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Alterations in the Circulation and Oxygen Consumption of the Liver Following Side-to-Side Portacaval Shunts and Eck's Fistula in Normal Dogs

Principal Investigator: Carlos R. Lombardo, M. D.

Other Investigators: Robert T. L. Long, M. D.
Fred Bullock
Henry Felton
Russell Holland

Man Years (calendar year 1958):
Total: 10/12
Professional: 6/12
Other: 4/12

Patient Days (calendar year 1958):
None

Project Description:

Hepatic arterial, portal venous, shunt and total hepatic blood flow has been measured in four normal dogs. The results indicate that both the oxygen consumption and hepatic blood flow are decreased following a shunting procedure.

In animals with side-to-side portacaval shunts the oxygen consumption and hepatic flow were lower than in animals with Eck's fistula. This is a result of retrograde flow of hepatic arterial blood via the portal vein - inferior cava shunt. The relative contributions of hepatic artery and portal vein flow to total liver blood flow and oxygen consumption will also be studied.

Proposed course of project: Continuation of the current project until a satisfactory number of dogs have been studied and finally to repeat this study in dogs with artificially induced cirrhosis and portal hypertension.

Part B included No.

Serial No. NHI-266
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: An Experimental Evaluation of the Use of Radioactive Gas Solutions in the Diagnosis of Left-to-Right Shunts

Principal Investigator: Robert T. L. Long, M. D.

Other Investigators: William Cornell, M. D.
Henry Felton
Samuel Fountain
Leander Brown

Man Years (calendar year 1958):
Total: 6/12
Professional: 3/12
Other: 3/12

Patient Days (calendar year 1958): None

Project Description:

The "left-sided" injection of radioactive gas solution in detecting and localizing left-to-right shunts was investigated in nine dogs with subclavian-pulmonary artery anastomosis. The radioactivity of the expired air was sampled, or recorded continuously by means of a direct writer. Aortic root injection of a solution of radioactive Krypton produces an expired air curve with an appearance time of 3 seconds and a short buildup time of approximately 11 seconds. When no shunt is present the appearance time is approximately 12 seconds and the buildup time 22 seconds. Proof that radioactivity has been injected is obtained by sampling from the femoral artery after injection. This technique is applicable in many situations where dye dilution curves have been used.

Proposed course of project: Preparation is being made to apply this technique in patients in the Cardiac Catheterization Laboratory.

Part B included No.

Serial No. NHI-267
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Pharmacologic Studies During Cardiopulmonary Bypass

Principal Investigator: John Ross, Jr., M. D.

Other Investigators: Eugene Braunwald, M. D.
John A. Waldhausen, M. D.
James A. McFarland, M. D.
Robert White
Robert Lewis

Man Years (calendar year 1958):
Total: 11/12
Professional: 2/12
Other: 9/12

Patient Days (calendar year 1958): None

Project Description:

In this investigation the heart-lung apparatus is used to obtain physiologic and pharmacologic information about isolated vascular areas while the balance of the circulation is hemodynamically stable. A rotating disc oxygenator and non-occlusive roller pump are used. Systemic output and femoral artery flow are recorded with a rotameter or an electromagnetic flowmeter.

The peripheral action of digitalis has been studied. The aorta is clamped above the coronaries to completely exclude any cardiac effects. A pressor effect of Ouabain has been consistently observed. The average maximum increase in total peripheral resistance is 60%, the peak effect occurring within 30 minutes. The pressor effect is not blocked by hexamethonium, although the average pressure increase is decreased. The above results have been consistent in the 10 dogs studied.

Proposed course of project: Further investigation is in progress to demonstrate more clearly the site of the pressor action of digitalis. A similar study using Cedilanid is planned. In addition, the study will be extended to determine the effects of digitalis glycosides on the pulmonary circulation.

Part B included No.

Serial No. NHI-268
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: An Experimental Investigation of Myocardial Metabolism during Cardiac Arrest

Principal Investigator: Joseph W. Gilbert, M. D.

Other Investigators: Robert T. L. Long, M. D.
Henry Felton
Russell Holland

Man Years (calendar year 1958):
Total: 6/12
Professional: 3/12
Other: 3/12

Patient Days (calendar year 1958):
None

Project Description:

Experiments have been performed in seven dogs using potassium citrate as the arresting agent and in three others using acetylcholine.

Cardiac arrest was induced with the arresting agent after caval occlusion. The aorta was then opened and the coronary ostia were cannulated. The heart was then perfused for twenty minutes with fresh heparinized arterial blood drawn from donor dogs. Flow rates were based on the flow obtained from the coronary sinus cannula before arrest. Determinations of oxygen, carbon dioxide, glucose, lactic acid, and unesterified fatty acids were made on the perfusate collected from the coronary sinus during arrest, as well as on the fresh blood in the reservoir. Oxygen utilization and carbon dioxide production, as well as consumption of the substrates glucose, lactate and unesterified fatty acids were then calculated. Following twenty minutes of arrest, resuscitation was attempted by perfusion with fresh arterial blood containing no arresting agent. Results to date indicate no oxygen or substrate utilization during potassium arrest, but diminished utilization during acetylcholine arrest. Consumption during ventricular fibrillation is approximately that of the beating heart. Resuscitation cannot be accomplished after continuous perfusion with an arresting agent for twenty minutes.

Proposed course of project: Plans for future-An extension of the present series, with more dogs arrested by acetylcholine is planned.

Part B included No.

Serial No. NHI-269
1. Clinic of Surgery
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Transeptal Catheterization of the Left Heart

Principal Investigator: John Ross, Jr., M. D.

Other Investigators: William Laughlin
Robert Lewis

Man Years (calendar year 1958):
Total: 6/12
Professional: 3/12
Other 3/12

Patient Days (calendar year 1958):
None

Project Description:

In the interval since the previous report, additional dogs have undergone left heart catheterization from the right heart using the retractable needle previously described. In addition, a somewhat larger needle was constructed, and a small catheter passed through it into the left ventricle in some animals. Through this needle, satisfactory left-sided angiocardiograms were obtained in nine dogs.

Proposed course of project: The experimental study has been completed. The procedure is being tested in cadavers and results indicate that its use in humans would be feasible.

Part B included Yes

Serial No. NHI-269

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B.

Article in Periodical:

Ross, J. Jr.: Transeptal Catheterization of the Left Heart: A New Method of Left Atrial Puncture. Annals of Surgery. To appear in February 1959 issue.

Serial No. NHI-270
1. Clinic of Surgery
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Hemodynamic Studies During Total Body Perfusion

Principal Investigator: John Ross, Jr., M. D.

Other Investigators: Eugene Braunwald, M. D.
John A. Waldhausen, M. D.
James A. McFarland, M. D.
Robert White
Robert Lewis

Man Years (calendar year 1958):
Total: 1
Professional: 3/12
Other: 9/12

Patient Days (calendar year 1958):
None

Project Description:

The general purpose of this project is to study the hemodynamic effects of perfusion under circumstances similar to those encountered clinically, and, in addition, to investigate the effects in isolated vascular areas of alterations in O_2 and CO_2 tensions. The initial area of study has been the hind limb of the dog.

A rotating disc oxygenator and a nonocclusive roller pump have been used. Total pump output is measured with either an electromagnetic flowmeter or a Potter turbinometer. A rotameter is used to record blood flow in the femoral artery. Oxygen tension is monitored with a Clark polarographic electrode.

Total systemic flow is maintained constant and alterations in femoral artery flow are measured during one-half hour perfusions in dogs. Immediately prior to perfusion, limb vascular resistance is very high. In general, during perfusion, there is initially an abrupt fall in limb resistance, followed by a gradual increase in both limb and total resistance during the remainder of the perfusion.

Part A. (continued)

Project Title: Hemodynamic Studies During Total Body Perfusion

Project Description:

Limb flow has also been recorded during hypoxia. The circulating blood is desaturated in the oxygenator using a 12% O_2 mixture and decreased disc rotation. With total flow maintained constant, limb and total resistance decrease markedly after 6 to 10 minutes of hypoxia; however, the initial response has been variable.

Proposed course of project: Further general perfusion and hypoxia studies are planned. It may prove more profitable to measure flow during perfusion in the renal or splanchnic areas; a separate study on hepatic blood flow during perfusion is in progress by one member of the group. It is planned to extend the hypoxia studies to the pulmonary vascular bed.

More adequate control of CO_2 levels is necessary, particularly in the hypoxia experiments. CO_2 and O_2 tension electrodes are under construction, at present, in connection with studies on membrane gas exchange being done in Dr. Robert Bowman's laboratory. These electrodes should prove useful in both studies. A joint study of the physiologic aspects of membrane oxygenator use is planned.

Part B included Yes

Serial No. NHI-270

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B.

Article in Periodical:

Ross, J. Jr., Gilbert, J. W., Sharp, E. H., Morrow, A. G.: Elective Cardiac Arrest During Total Body Perfusion: The Relationship of Elevated Intracardiac Pressures During Arrest to Subsequent Myocardial Function and Pathologic Pulmonary Changes. Journal of Thoracic Surgery, Vol. 36, No. 4, pp. 534-542, October 1958.

Serial No. NHI-271
1. Clinic of Surgery
3. Bethesda

FHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Elective Cardiac Arrest During Total Body Perfusion in the Experimental Animal. The Relationships of Elevated Intracardiac Pressures to Myocardial Failure and Pathologic Pulmonary Changes

Principal Investigator: John Ross, Jr., M. D.

Other Investigators: Joseph W. Gilbert, M. D.
Edward H. Sharp, M. D.
Andrew G. Morrow, M. D.
Russell Holland
Ray Waters
Robert White
William Laughlin

Man Years (calendar year 1958):
Total: 9/12
Professional: 3/12
Other: 6/12

Patient Days (calendar year 1958)
None

Project Description:

In a significant percentage of animals and patients subjected to total cardiopulmonary bypass and elective cardiac arrest, pulmonary congestion is seen in the postoperative period. It was postulated that this was due to elevated pulmonary venous pressure secondary to the technique of arrest. Utilizing the Melrose pump oxygenator, 31 dogs were studied by the continuous measurement of right ventricular, aortic and left atrial pressures throughout a period of cardiac perfusion, arrest and recovery. Histologic study of the lungs was performed before and after perfusion. Control dogs without cardiac arrest or cardiotomy showed no pressure elevations in the cardiac chamber. Cardiac failure and lung damage did not occur in this group. Similar results were obtained in those animals in which a continuous atriotomy was maintained throughout arrest and recovery. However, in dogs subjected to cardiac arrest without cardiotomy or with cardiotomy late in the period of arrest, significant rises in left atrial and right ventricular pressures were noted. In addition, these animals showed complications

Serial No. NHI-271

Part A. (continued)

Project Title: Elective Cardiac Arrest During Total Body Perfusion in the Experimental Animal. The Relationships of Elevated Intracardiac Pressures to Myocardial Failure and Pathologic Pulmonary Changes

Project Description:

such as ventricular fibrillation and cardiac failure during the recovery period. In the lungs of some of these animals microscopic resections revealed edema and hemorrhage. It was found that these pressure increases were due to the pulmonary venous return secondary to bronchial artery flow and that the pressure elevations and consequent pulmonary pathology could be prevented by cardiotomy. It was significant that elevation of pulmonary venous pressure could be prevented by right-sided cardiotomy as well as by left-sided cardiotomy, and that intracardiac pressure changes were magnified by simultaneous occlusion of the pulmonary artery and aorta. It was shown that occlusion of the pulmonary artery prevents the escape of bronchial artery flow into the right heart through the collapsed and incompetent pulmonary valve.

Part B included Yes.

Serial No. NHI-271

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B.

Article in Periodical:

Ross, J., Jr., Gilbert, J. W., Sharp, E. H., Morrow, A. G.: Elective Cardiac Arrest During Total Body Perfusion: The Relationship of Elevated Intracardiac Pressures During Arrest to Subsequent Myocardial Function and Pathologic Pulmonary Changes. Journal of Thoracic Surgery, Vol. 36, No. 4, pp. 534-542, October 1958.

Serial No. NHI-272
1. Clinic of Surgery
2. Section on Cardiology
3. Bethesda

PHS - NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: A Study of the Distensibility of the Canine Ventricle During Diastole

Principal Investigator: Eugene Braunwald, M. D.

Other Investigators: John Ross, Jr., M. D.
Robert L. Frye, M. D.
Robert Lewis

Man Years (calendar year 1958):
Total: 10/12
Professional: 6/12
Other: 4/12

Patient Days (calendar year 1958):
None

Project Description:

The purpose of this study is 1) to describe the relationship between end-diastolic pressure and end-diastolic fiber length of the left ventricle of the dog, i.e., establish a pressure-volume curve for the relaxed ventricle, 2) to determine whether the pressure-volume curve can be modified by interventions such as catechol amine infusion, alterations in aortic pressure, heart rate, and changes in myocardial contractility, and 3) to study Starling's law of the heart by observing the relationship between end-diastolic fiber length and stroke work.

The major effort to date has been devoted to developing methods for the measurement of end-diastolic fiber length and volume. This has been accomplished by means of a modified Cushny lever which is sewn on the surface of the left ventricle and permits measurement of end-diastolic fiber lengths of a segment of cardiac muscle. End-diastolic volume is determined at the end of the experiment after the dog has been sacrificed by adding known volumes of fluid to a balloon placed within the left ventricle and by relating the Cushny lever deflection to the volume within the balloon and left ventricle. Distensibility is determined by stepwise blood infusions and the recording of both end-diastolic fiber length and end-diastolic pressure after each infusion.

Part A. (continued)

Project Title: A Study of the Distensibility of the Canine Ventricle During Diastole

Project Description:

There have been five technically satisfactory experiments. The distensibility curve of the ventricle exhibits a flat portion, in which there is a large volume change associated with a small pressure change. This is followed by an inflection at an end-diastolic pressure in the neighborhood of 15 cm. H_2O and a steep portion of the curve at higher end-diastolic pressures. This shape has been observed quite consistently. Ventricular distensibility determined during the infusion of epinephrine or nor-epinephrine has not been significantly different from the control state. It is apparent that the catechol amines also result in greater stroke volume and stroke work for any given end-diastolic volume. This would indicate that the inotropic action of these drugs is effected by more complete systolic emptying.

Immediately after death, distensibility has not been observed to be significantly different from during life, suggesting that ventricular relaxation is complete during diastole.

Constriction of the pericardium has been noted to diminish diastolic distensibility. In two experiments, a progressive increase in diastolic distensibility occurred as the experiment progressed. In one of these, epinephrine shifted the pressure-volume curve back to control levels.

Proposed course of project: Additional experiments will be carried out on the effects of catechol amines. Aortic pressure, heart rate, anoxia and sympathetic stimulation will also be studied.

Part B included No

Form No. OSP-2
Oct. 1957

PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
Laboratory of Technical Development

Estimated Obligations for FY 1959

Total: $272,760

Direct: $207,000

Reimbursements: $ 65,760

Serial No. NHL-273
Laboratory of Technical Development
Bethseda 14, Maryland

PHS-NIH
Individual Project Research
Calendar Year 1958

Part A.

Project Title: Development of an Ultramicroanalytic Method for Sodium and Potassium Determination in Micropuncture Samples

Principal Investigator: Robert L. Bowman

Other Investigators: Edmund S. Hoffmaster (High School Teacher)
Bruce Livingston (summer)

Cooperating Units:

Edmund S. Hoffmaster (High School Teacher)
Dr. John R. Jaenike (LKEM)

Man Years (calendar year 1958)
Total: .48
Professional .08
Other: .4

Patient Days - None

Project Description:

Progress During Past Year:

During the development of the gas discharge detector for gas chromatography, it was noted that the central electrode would glow with a bright yellow color indicating the presence of sodium. It appeared that the helium was very sensitive in ionizing alkali metals on the tip of the wire or carried into the gas stream in a volatile state. Various arrangements were investigated for providing satisfactory discharge and sample handling. A system utilizing a .006" diameter platinum wire as the sample carrier was chosen. Methods for loading the wire with a measured quantity of sample were evaluated utilizing fluorescence indicators. No satisfactory method for picking up a reproducible quantity was found. The sample was therefore deposited directly on the wire from a capillary observed microscopically. The background spectrum is a line spectrum with no continuous radiation as found in flames. The integrated output of sodium light is proportional to the quantity of sodium introduced and essentially all of the sodium contributes to the emission since the gas flow is so much lower than in flames. The helium yellow line is

Progress During Past Year (continued)

very close to the sodium line, but no difficulty was encountered in separating them with a Bausch and Lomb grating monochromator. The apparatus has been turned over to Dr. John R. Jaenike for evaluation of its utility for biological samples. Preliminary results indicate 10^{-12} moles of sodium as a working level.

Direction of Current Research:

The rare gas discharge activation of alkali metal emission will be studied further to establish its ultimate sensitivity and dependability with an ultimate aim of an instrument capable of analyzing these materials in a range of quantity available by micro-puncture techniques.

Part B - included - NO

Serial No. NHI-274
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: The Biochemical Effects of Ultrasonic Waves

Principal Investigator: Alfred Weissler

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1958):
Total: .8
Professional: .8
Other None

Patient Days(calendar year 1958) None

Project Description:

Progress During Past Year: (Began July, 1957)

The purpose of this research is to elucidate the detailed processes by which ultrasonic waves affect living tissues and simpler biochemical systems such as enzyme solutions. In order to study the role of free radicals in chemical reactions caused by ultrasound in aqueous systems, the sonochemical yield of hydrogen peroxide was measured in water which contained radical scavengers in various concentrations and was also saturated with either oxygen or argon. The results indicate that hydroxyl radical is an intermediate produced from water by ultrasound; this radical and perhydroxyl radical (formed in oxygenated solutions) are considered principally responsible for such sonochemical effects as oxidation and polymerization.

In a general way, this resembles the situation in ionizing-radiation chemistry, but there are notable differences in detail. Quantitative comparison of ultrasound and gamma-rays, with respect to the amount of chemical change caused per unit of energy absorbed, in three different reactions, showed that ultrasound is several hundred time less efficient. The three reactions investigated were: the oxidation of 0.001 M ferrous sulfate in 0.4 M sulfuric acid (widely used for dosimetry in radiation chemistry); the reduction of 0.0001 M ceric sulfate in 0.4 M sulfuric acid; and the formation

of hydrogen peroxide in oxygen-saturated distilled water. The measurements were made in a solution volume of 250 ml, contained in a polythene bag with a wall thickness of 0.001 inch, immersed in 100 ml. of degassed distilled water contained in a focussing barium titanate transducer assembly operating at 400 kilocycles. The solution was exposed to ultrasound for 5.0 minutes, at a power level of 67 acoustic watts as determined by a colorimetric substitution method. The ratio of efficiencies of ferrous ion oxidation to ceric ion reduction (which is a measure of the Linear Energy Transfer of ionizing radiation) indicates that ultrasound is equivalent in "quality" to very low energy alpha-particles.

Some preliminary work was done on the ultrasonic irradiation of urease solution. Urease was chosen as a suitable enzyme because it has both a high molecular weight and a sensitivity to oxidation due to its many sulfhydryl groups. The urease available, however, had low activity; consequently, little destruction was observed because of the protective action of the large amount of inert material. Purification attempts did not greatly increase the specific activity. It will be necessary to obtain a better sample of urease of other sulfhydryl enzyme.

Direction of Current Research:

Solutions of enzymes and other proteins will be treated with ultrasound and the loss of activity will be correlated with spectrophotometric and molecular weight changes; comparisons will be made with similar published work in radiation chemistry. The effect of washing dissolved air out of the solutions, prior to treatment, with gases such as argon or oxygen will be studied, with a view to decreasing the amount of enzyme destruction encountered during ultrasonic extraction of enzymes from tissue homogenates. Also under consideration is work on functional damage to protozoa exposed to moderate-intensity ultrasound.

Part B included - Yes

Serial No. NHI-274

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

"Formation of Hydrogen Peroxide by Ultrasonic Waves: Free Radicals", accepted for publication in February, 1959, by Journal of the American Chemical Society.

Serial No. NHI-275
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Development of Methods for Measurement of Blood Flow

Principal Investigator: Robert L. Bowman

Other Investigators: Vsevolod Kudravcev - CC
(Dr. Murray Brown's section)
Claude M. Weil Malherbe(summer)

Cooperating Units:

Vsevolod Kudravcev - Clinical Center (Dr. Murray Brown's section)
Mr. Kudravcev has been the main engineer designing and constructing the improved NMR apparatus.

Man Years (calendar year 1958):
Total: 1.15
Professional: .04
Other: .95

Patient Days: - None.

Project Description:

Progress During Past Year:

The aim of this continuing project is to produce an efficient method of measuring blood flow with a minimum disturbance of the system being measured. The method utilizing nuclear magnetic resonance is still considered to have the best promise for fulfilling the requirements. Previous experience had shown the amplitude of the resonance signal to be erratic so that a flow curve could not be produced within better than 20%. It was presumed that the flow also detuned the oscillator so that it was necessary to provide some form of automatic frequency control. This thesis was tested by running flow curves and retuning the system for each reading. These flow curves showed an excellent smooth curve and were highly reproducible over the range of interest. On the basis of manual returning experience the

Progress During Past Year (continued)

apparatus is being rebuilt with automatic frequency control and several other electronic regulatory systems. It was interesting to note that the flow curves showed a break in the slope which appears to arise at the time the stream breaks into turbulent flow. The region of the break is compatible with Reynold's number calculations for the turbulent point, but a more dependable confirmation will have to be made. The development of the apparatus for measurement of flow has so much in common with the apparatus for broad line NMR analysis that the new apparatus is being designed to allow both of these projects to utilize the same equipment.

Direction of Current Research:

As soon as it is currently shown that the NMR signal will measure continuous flow, steps will be taken to investigate the possibility of using the induced nuclear precession as a marker to trace and measure flow; attempts will be made to determine whether or not the tissue water can be distinguished from the water flowing in the vascular bed; the possibility of measuring the alkali metals in entire small animals and the general problem of broad line measurements evaluated.

Part B included - NO

Serial No. NHI-276
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Aqueous Scintillator Solution for Carbon-14 Counting

Principal Investigator: Alfred Weissler

Other Investigator: None

Cooperating Units: (Supported by Laboratory of Cellular Physiology and Metabolism (Dr. Steinberg).
Equipment - Packard Automatic Scintillation Counter
Personnel - Walter Lewis (technical)

Man Years (calendar year 1958):
Total: .2
Professional: .2
Other: None

Patient Days(calendar year 1958)
None

Project Description:

Progress During Past Year: (Began October, 1958)

The purpose of this research is to develop a method for scintillation counting of C^{14} in aqueous solution, in order to avoid the inconvenience of counting aqueous samples in toluene solution as at present. Work by others has shown that fluorescent materials in aqueous solution do not count beta-particles; therefore, the use of chemiluminescence is being studied. The most familiar chemiluminescent substance, "luminol" (3-amino-phthalhydrazide), will emit light in alkaline solution when exposed to ultrasound, presumably as a result of oxidation by hydroxyl radicals or hydrogen peroxide. Similarly, the hydroxyl radicals or hydrogen peroxide produced by beta-particles should cause light emission in luminol solutions.

The amount of light emitted, as a function of luminol concentration and sodium hydroxide concentration, has been investigated with the Tri-Carb automatic scintillation counter. In some cases, apparent counting efficiencies as high as 50% have been found, but the results thus far are not sufficiently reproducible. Unlabeled solutions show a high variable background count, which seems to be affected by a variety of factors such as age, oxygenation, temperature, freezing and previous illumination. Hydroquinone acts as a quencher, and hemoglobin acts as an intensifier. Pulse height analysis of blank versus labeled solutions does not reveal striking

differences. Light emission drops to a low value after a day or two in the most dilute solutions (0.000001% luminol) which indicates that the luminol is slowly destroyed. Washing out the dissolved air with nitrogen does not greatly reduce the light emission.

Other chemiluminescent materials are known, such as lucigenin (dimethylbiacridinium nitrate), triaminosiloxene, and violanthrone dye. None of these is both (a) water-soluble and (b) an indicator of hydroxyl or hydroperoxyl radicals; therefore our studies thus far have been concentrated on luminol.

Direction of Current Research:

Attempts are being made to lower the background count and to increase the reproducibility. Solutions containing both hemoglobin and hydroquinone are under study, in order to find whether a favorable balance point exists between the intensifier and quencher effects. If satisfactory counting of carbon-14 is achieved, the work will be extended to tritium and to solutions containing easily oxidized substances such as sulfhydryl groups.

Part B included - No

Serial No. NHI-277
Laboratory of Technical Development
Bethseda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Development of Micro Glass Electrodes for the Measurement of pH and of Sodium Concentration

Principal Investigator: Murray Eden

Other Investigator: Laura Guiffrida

Cooperating Units:

Dr. Jack Jaenike Dr. George Bray Dr. Robert W. Berliner - LKEM

Man Years (calendar year 1958) Patient Days - None
Total: 1.5
Professional: .5
Other: 1

Project Description:

Progress During Past Year: (Project began June 1955)

Further attempts have been made to coat the electrodes with an insulating film. So far the best results have been obtained with a propietary varnish furnished us by Beckman Instrument Company. With this varnish electrodes may be coated effectively to within approximately 300 microns of the tip.

Extensive tests have been performed on the alkali metal ion sensitive glasses described by Eiseman et. al. Micro electrodes of a suitable glass composition have been made which are sensitive to sodium and are only 1/9th as sensitive to potassium. The electrodes are also insensitive to pH in the usual physiological range. These electrodes exhibit a response very close to theoretical ie. a 58 millivolt potential change is induced by a tenfold change in sodium activity.

Direction of Current Research:

Preliminary investigations of the changes in sodium concentration in dog kidneys during the course of various experimental procedures have suggested that the electrodes may be useful in determining interstitial electrolyte concentrations in various tissues in vivo. These researches which have been conducted in collaboration with workers in LKEM will be pushed further as an additional tool for the explication of the kidney concentrating mechanism.

Progress During Past Year (continued)

Further development will be attempted to obtain electrodes preferentially sensitive to potassium and to calcium. Although the latter will in all likelihood require a somewhat different approach.

The efforts to develop true micro electrodes coated to within about 50 microns of the top will be continued.

Part B - included - NO

Serial No. NHI-278
Laboratory of Techincal Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Development of Freezing Point Depression Methods

Principal Investigator: Robert L. Bowman

Other Investigator: Christopher Eve

Cooperating Units: None

Man Years (calendar year 1958): Patient Days - None
Total: 1.0
Professional: .2
Other: .8

Project Description:

Progress During Past Year:

A system for observing several droplets of standards and unknowns while undergoing freezing and thawing was set up. The samples consisted of from 2 to 3 micro-milliliters placed separately under oil in 8 holes in a copper block. The entire circle of holes being placed within the field of a microscope so that they can be observed under magnification of 50 to 100 times. The copper block is then cycled over a range of temperature to rapidly freeze and slowly thaw the samples. On the assumption that all of the droplets experience the same temperature change and that they are each too small to influence the temperature. The time of thawing is used as an index of melting point. Rapid cooling and slow warming have been accomplished effectively and reproducibly by use of a chilled block of metal that is placed over the sample holder. Warming rates of the order of 2 "milliosmoles per second" have been used and the system evaluated for standard solutions. Freezing points determined in this way have exhibited an error of approximately ± 8 milliosmoles. The error does not seem to be any different for low as compared to high concentrations. Several methods of reducing this error are yet to be tested but the existence of an effective method (Ramsey's) makes continued effort at this level of questionable value. We have demonstrated

Progress During Past Year (continued)

that there is a finite possibility of using this system to determine freezing points of even smaller quantities, but the application should wait until the apparatus can be evaluated on an actual problem.

Direction of Current Research:

To continue to devise and test new methods and instruments as the occasion arises to apply them to local research programs.

Part B - included - No

Serial No. NHI-2/9
Laboratory of Technical
Development
Bethseda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Design and construction of a more sensitive detection device for vapor phase chromatography and development of methods for the microanalysis of fatty acids

Principal Investigators: Arthur Karmen
Robert L. Bowman

Other Investigators: Willard Whitehouse
Harold Tritch(summer employee)

Cooperating Units: None

Man Years (calendar year 1958): Patient Days = None
Total: 2.4
Professional: 1.6
Other: .8

Project Description:

Progress During Past Year:

Objectives: To design a more sensitive vapor detector to permit the use of small samples of fatty acid esters in gas chromatography, samples in the range usually obtainable from biologic materials, and to obtain the increase in column-efficiency of separation obtainable by the use of smaller samples.

A review of the literature at the onset of this study revealed that the most sensitive detectors that had been described were based on measurement of the electric characteristics of the effluent gases. Ionization of the gas in an ionization gauge using helium as carrier gas was reported to permit quite sensitive detection of hydrocarbon vapors. An apparatus of this type was constructed and it was found that passage of hydrocarbon vapor through the tube elements contaminated and destroyed these elements and stability sufficient for use with gas chromatography was not obtainable. Experiments with corona discharge in helium at atmospheric pressure and at reduced pressure revealed similar destruction and change of the tube elements and instability of the discharge. By using radio frequency voltages to power the discharge, a sufficiently stable discharge could be obtained. The properties of radio frequency

Progress during Past Year (continued)

excited glow discharges in helium at an atmospheric pressure were studied, and experiments performed to determine what apparatus could best be used to power the discharge and cause it to have sufficient stability to permit its use as a detector.

A stable discharge could be excited in helium at atmospheric pressure by means of using a stabilized source of radio frequency power. Sufficient stability of this power source could be obtained by crystal control of the frequency and using regulated direct current power supplied to power the RF oscillator. A direct current potential is measurable across the plasma of an RF excited discharge. Presence of a vapor other than helium in the discharge tube causes a diminution in this direct current. When organic vapors of many types are present in the helium, the decrease in the direct current is directly proportional to the concentration of the hydrocarbons in the helium. The direct current obtainable and the signals produced by the passage of hydrocarbons through the discharge was sufficient to permit recording on a standard recorder without additional amplification. The direct current output was found to increase with increase in temperature making the device more sensitive at higher temperature, and permitting its use in high temperature gas chromatography. Sensitivity to changes in temperature pressure and gas flow rate was not sufficient to require extraordinary care in stabilizing these factors.

Determination of the ultimate sensitivity of this device has not been possible because of the difficulties in presenting the discharge with a known precisely small enough concentration of hydrocarbon in the helium. Sensitivities have been obtained repeatedly in the order of 1 molecule of methyl myristate in 10^6 molecules of helium sufficient to obtain complete analysis of long chain fatty acids using a sample volume of 10^{-6} ml. Sensitivity is at least 1000 times greater than therma l conductivity detectors.

The detector is simple in construction and has been adapted for use with two conventional, commercially available chromatographs, with increase in their sensitivity, in the resolving power of their columns caused by using smaller samples, and is rugged and stable enough for several months of continuous use without difficulty.

Description: Ancillary methods for the microanalysis of fats in preparing samples for gas chromatographic analysis of constituent fatty acids: 1) sample injector - to make possible the introduction of microgram quantities of liquid samples in precisely knwosn amounts into the chromatographic column without contaminating the column and the analysis with large quantities of carrier liquid, a sample introduction device was constructed. Microgram quantities were sealed in small glass capillaries, their volume determined with a measuring microscope, and a device constructed for introducing the capillary and crushing it at the column inlet without interruption of the flow of carrier gas.

Progress during Past Year (continued)

2) Techniques for the recovery of small samples from high dilution with solvents - when methyl esters of fatty acids in quantities less than several hundred micrograms are prepared in glass vessels, collection of the esters in form suitable for introduction to the gas chromatography becomes difficult because of the property of these esters of forming semi-solid films on the walls of the container when the solvent in which they are dissolved is evaporated. Techniques have been devised for their collection in a glass capillary by a micro-refluxing technique and devices for the introduction of these capillaries into the chromatograph have been constructed. 3) Group separation of lipids by silicic acid paper chromatography - methods for the separation of lipid fractions by silicic acid column chromatography have been developed. The size of the sample required is usually more than that available from small samples of biologic materials, and the analyzed materials are presented in high dilutions of solvents whose purity by the standards of gas chromatography is not high. Accordingly, a method was developed for group separations of lipids on silicic acid coated paper using samples of small size compared to those used in column chromatography and larger than the samples usually used with paper chromatography. It was found possible to separate the cholesterol esters, glycerides, and phospholipids in 0.5 ml. of plasma determine their location on the paper chromatogram by a simple, sensitive detection technique esterify the fatty acids present, collect the esters and analyze them by gas chromatography. This paper chromatographic technique holds promise of being applicable to other studies in which rapid separation of these lipid groups is required.

Significance to Heart Research - Gas chromatography with the use of this detector makes possible microanalysis of the fatty acids in the quantities usually obtainable from small blood samples and thus makes possible studies of serial changes in small biologic samples of this type. It is hoped that studies utilizing these methods of analysis will lead to better understanding of the metabolism of fatty acids and fats and will delineate the relationship between fat metabolism and the development of atherosclerosis and other disorders.

(b) Direction of Current Research:

The projects described represent design of a complete system for the microanalysis of fatty acids. It is intended to apply these methods to the determination of the fatty acids in the different lipid fractions of blood, and to study their metabolism and changes as influenced by diet in healthy and diseased individuals.

Part B included - Yes

(Attachment I)

Serial No. NHI-279

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications:

A micro sample introduction system for gas chromatography Robert Bowman and Arthur Karmen, Nature, Vol. 182, pp.1233-1234, Nov. 1, 1958.

A Radio Frequency Glow Detector for Gas Chromatography, Arthur Karmen and Robert L. Bowman, New York Academy of Sciences Symposium on Gas Chromatography, in press.

Serial No. NHI-280
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Machine Analysis of Absorption Spectra

Principal Investigator: Joseph E. Hayes, Jr.

Cooperating Units:

Spectra are determined in a Cary Model 11 Spectrophotometer in the Laboratory of Natural Products, National Heart Institute.

Man Years (calendar year 1958): Patient Days - None
Total: .1
Professional: .1
Other:

Project Description:

Progress During Past Year: (Began 1958)

The absorption spectra of organic compounds depend on the structure of the absorbing molecule. Only in the infra-red region have extensive correlations of this kind been found. Numerous attempts have been made to find such correlations in visible and ultraviolet spectra, which arise from electronic transitions in the absorbing molecules and which are much more poorly resolved. These attempts have resulted in several rules of thumb which amount only to a naming of complexes of absorption bands and to a prediction of whether substitution of a given group into the molecule will result in the complexes shifting to longer or to shorter wavelengths.

A ready rationalization of the failure of such attempts to yield more information is found in the extreme techincal difficulty of resolving these complexes into the individual absorption bands. A computer designed in this laboratory(See A Computer for Determining Wave Number, Intensity and Line Width of Maxima in the Infra-Red Spectrum 1958 Annual Report) furnishes a ready method of analyzing such spectral band complexes and it is expected that this will permit determination of the parameters of the individual absorption bands so that correlations with structure can be made. It is hoped that this will yield an independent method of evaluating the importance of various "contributing forms" in a resonance sense.

Progress During Past Year (continued)

To date, analyses have been made of the absorption spectra of 2, 4 - dinitrophenol and the three isomeric mononitrophenols in buffered aqueous solution in the region 20,000 - 50,000 cm^{-1}. These indicate that there is indeed a definite systematicity in the sprectra, but from such a smallnumber of compounds nothing definitive can be shown. A series of aryl nitro compounds, a group selected because of its readyavailability, comparatively simple spectra, and fairly well understood resonance behavior, have been obtained and purified. Further compounds, such as the other five position isomers of 2, 4 - dinitrophenol, have been prepared.

Direction of Current Research:

The spectra of these compounds, some seventeen in all, in nonaqueous, acid aqueous, and alkaline aqueous solution, should permit assessment of whether the correlations found are useful. If so, they will be extended to compounds of greater intrinsic interest.

Part B included - NO

Serial No. NHI-281
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Drying and Hydration Studies of Proteins and Tissue

Principal Investigator: John L. Stephenson

Other Investigators: Geraldine W. Smith
Artrice Valentine
Marion Sharpless

Cooperating Units: None

Man Years (calendar year 1958): Patient Days - None
Total: 1.2
Professional: .3
Other: .9

Project Description:

Progress During Past Year: Began 1954

The general purpose of this project is to obtain information on the nature of the solid-liquid interface in protoplasm and particularly its area and hydration structure, also to obtain empirical information on the drying of various biological materials to aid in the rational design of drying apparatus.

This year the compilation of experimental drying data with previously described vacuum balance (Stephenson, Smith, Tranthan, Rev. Sci. Inst. 28: 381-382) has continued, and at least for the present has been completed. Materials whose vacuum drying rate from the frozen state has been measured include 10 percent bovine serum albumen, 2 percent ova-albumin, 10 percent ova-albumin, blood plasma, 2 percent gelatin and guinea pig liver. Utilizing a theory first developed by Derjaguin, (Compt. Rend. Acad. Sci. U.R.S.S. 53:623 (1946), which we have modified and extended, we have computed surface area per gram dry weight for the various materials from these drying data. With the exception of gelatin, these area have been much less than those computed either from supposed molecular dimensions or from gas absorption data. The reason for this is that during the freezing there is great precipitation and clumping of the material (this is obvious on microscopic

Progress During Past Year (continued)

examination) and this method of measuring surface area measures the surface of the envelope of the clumps rather than the surface enveloping individual molecules.

This work was described in April at the Second International Conference on Freezing and Drying held in London. It has also been written up and submitted for periodical publication.

Direction of Current Research:

No further work during the next year is planned on this project.

PART B Included - NO

Serial No. NHI-282
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Physics of Ultra-Rapid Freezing of Water, Colloidal Solutions and Protoplasm

Principal Investigator: John L. Stephenson

Other Investigators: Geraldine W. Smith
Artrice Valentine
Marion Sharpless

Cooperating Units: None

Man Years (calendar year 1958): Patient Days - None
Total: 1.5
Professional: .3
Other: 1.2

Project Description:

Progress During Past Year: Began 1954

The general purpose of this project remains: (1) To investigate the basic physics of the rapid freezing process in water, colloidal systems and protoplasm. (2) To apply this information to the analysis of hydration phenomena in protoplasm. (3) To extend the range of application of freezing and drying as a method of fixation and preservation of biological material.

Work on electron microscopy has continued and the routine problems of specimen preparation and operation of the electron microscope seem to have been largely solved. The satisfactory embedding of frozen dried material still presents a problem. One scheme, developed in collaboration with Dr. Fritioff Sjostrand of the Karolinska Institute, during a visit to his laboratory this spring, shows some promise. In this methyl methracrylate is distilled over, under vacuum, into a cold finger containing the dried specimens. These specimens are then transferred to individual tubes and further prepared for electron microscopy as described by Sjostrand and Baker (J. Ultrastructure). The

Progress During Past Year (continued)

use of frozen dried material for electron microscopy presents two fundamental problems: One is that embedding materials do not seem to completely infiltrate frozen dried tissue, possibly because of failure to completely wet the tissue which still has some residual water of hydration no matter how long it is dried. The other is that frozen dried material without subsidiary fixation does not stand up under the electron beam. Both of these problems will obviously limit the use of frozen dried material for critical ultrastructure studies, but on the other hand rapid freezing offers possibilities of correlating ultrastructure with momentary physiological states which no other method does. One problem in evaluating fixation procedures is the cutting of sufficiently small specimens and also knowing whether these represent protoplasm in a more or less the same physiological state. To try to solve this problem we have started colonies of both amoeba Chaos Chaos and of paramecia.

Further experimental work has been done evaluating the properties of various coolants--in particular, the Freons, which have been found to be nearly as good coolants as isopentane or propane and are much less hazardous to use. The primary purpose of these and earlier experiments has been to analyze the conditions of heat transfer at the surface of the sample being cooled. It has been found that to a good approximation heat transfer per unit area at the surface is given by $H(T_s - T_f)$, where T_s is the surface temperature and T_f is the temperature of the coolant. Theoretically H depends on a large number of factors, such as the velocity of the sample relative to the coolant, its size, shape, as well as the intrinsic properties of the coolant. Experimentally we have found it to be nearly a constant for a given coolant, e. g. $H = 0.2$ cals/cm^2. sec. deg. for propane and 0.02 cals/cm^2 sec. deg. for liquid nitrogen. If H is known for a coolant it is possible to develop an exact theory for the cooling of a solid of regular shape without phase change and an approximate theory for cooling with phase change. By application of the theory to experimental cooling curves it can be determined whether a phase change has occurred. Such an analysis of cooling curves of 10 percent gelatin blocks has indicated that for cubes less than 1 mm on edge, cooled in liquid propane there is only partial freezing of the water.

Direction of Current Research:

The primary problem in the next few months is a correlate size, number and occurrence of ice crystals as observed by electron microscopy with the calorimetric studies described above.

Part B included - NO

Serial No. NHI-283
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Development of a Probabilistic Model for Growth

Principal Investigator: Murray Eden

Cooperating Units: None

Man Years (calendar year 1958) Patient Days - None
Total: .1
Professional: .1
Other:

Project Description:

Progress During Past Year: (Project begun prior to arrival on duty June, 1955)

Better estimates of the number of possible forms have been computed. Further than this no progress has been made.

Direction of Current Research

Methods are being explored for preparing a more efficient Monte Carlo process of generating a sample of concentrations of large size by machine computation.

Part B - included - Yes

(Attachment I)

Serial No. NHI-283

PHS - NIH
Individual Project Report
Calendar Year 1958

Part B: Honors, Awards, and Publications

Publications:

A Probabilistic Model for Morphogenesis, in Symposium on Information Theory in Biology, Pergamon Press, Inc., 1958, pp. 359-370.

Serial No. NHI-284
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1958

Part A.

Project Title: Studies on Gas Transfer Through Artificial Membranes

Principal Investigators: John Ross
Eugene Booth

Other Investigator: Alexander McInnes

Cooperating Units:

1. Dr. Eugene Booth is Professor of Physics at Columbia University currently on leave of absence doing investigative work for the Navy Department. The initial theory and the plans for the oxygenator were largely of his design; he is at present acting in an advisory capacity on the project.

2. Oxygen determinations are performed in the Laboratory of the surgical division, NHI.

Man Years (calendar year 1958): Patient Days - None

Project Description: Total: 0.8
Professional: 0.6
Other: 0.2

Progress During Past Year:

The general purpose of this investigation is to study the factors affecting the efficiency of the oxygenation of blood through currently available membranes, with the ultimate aim of designing a practical oxygenator. Theoretical considerations suggested that at a minimum practical blood film thickness of .004" to .005", the plasma layer would limit diffusion rather than the membrane.

A small testing unit having a film area of 500 cm^2 has been constructed. 3/8 and 1/2 mil Teflon membranes have been studied with blood films of .005" and .009". In addition, preliminary studies have been performed using a .005" blood film between twin membranes, giving an effective blood film thickness of .0025". Fresh, positive serology human blood has been used. Temperature, pH, and oxygen content by Van Slyke or Beckman analysis are measured, and from these data oxygen tension gradients can be calculated. A single layer of 1/2 mil Teflon with a .005" blood film has a diffusing capacity of 0.036 (0.032-0.039) cc of $O_2/m^2/min/mm.Hg.$ mean gradient. This is approximately 25 percent more oxygen transfer than is obtained with the Clowes membrane oxygenator.

Serial No. NHI-284

Progress During the Past Year (continued)

An additional 18 percent increase results when 3/8 mil Teflon is used, indicating that at this blood film thickness, the membrane is still a limiting factor. Initial studies with the double membrane unit using 1/2 mil Teflon show approximately 100 percent increase in oxygen transfer over that obtained with a single layer of 1/2 mil film.

Direction of Current Research:

An apparatus for maintaining constant temperature and mixing is under construction. This will accommodate electrodes for direct measurement of O_2 and CO_2 tensions. This type of measurement should permit the use of plasma on another blood substitute during testing. Refinements in the technique of using twin membranes are in progress in an attempt to overcome the difficult problem of maintaining uniform blood channels.

Other materials, such as silicone rubber films, will be investigated. In addition, a joint project with the surgical unit is planned in the animal laboratory to study the physiologic aspects of membrane oxygenation.

Part B included - NO

Serial No. NHI-285
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Research
Calendar Year 1958

Part A.

Project Title: An Analog Computer for the Analysis of Overlapping Absorption Spectra

Principal Investigators: Frank W. Noble
Murray Eden
Joseph E. Hayes, Jr.

Cooperating Units: None

Man Years (Calendar Year 1958): Patient Days - None
Total: 2.1
Professional: 2.0
Other: 0.1

Project Description:

Progress During Past Year: Began March 1957

It has been shown that curves from a variety of experimental procedures, including absorption spectra, electrophoresis ultracentrifugation and diffusion, are capable of being analyzed into their components by an analog computer which generates a set of the appropriate distribution function, each function in the set being independently variable as to position, height and variance.

The first model has been developed as far as the basic design permits. The precision of the generating triangle is not as good as desired, and a rather long time delay occurs following width adjustments. The general stability is not as good as would be desired because the components are not the best obtainable. It has been found that a five channel instrument is not adequate for fitting some absorption spectra. It has been decided that a minimum of ten channels are required in order to fit the majority of curves. A new model has been designed and a single channel built and tested. The precision is very much improved, the time delay completely eliminated, and the stability improved through the use of superior components. A ten channel instrument of the new design is currently under construction in the NIH Instrument Section.

Progress During Past Year (continued)

A device has been constructed for projecting the trial solutions of the computer onto the experimental curve. This method eliminates many of the problems of calibration and scale since the solutions may be read off the coordinates of the experimental presentation.

Collaborative work has been carried on with Dr. Alfred Chanutin of the University of Virginia on electrophoretic patterns from lysates of aged red blood cells and with Mrs. Rebecca Parker of National Bureau of Standards on diffusion patterns from quaternary ammonium salt systems, which diffuse in a quite anomalous fashion.

A ten channel analog computer of the second type has been constructed by the central shop for use by Dr. F. Brackett's group in the Laboratory of Physical Biology of NIAMD.

Direction of Current Reasearch:

A series of synthetic overlapping distributions has been prepared and will be analyzed by both the phototype instrument and the new model in order to evaluate quantitatively the accuracy of the device under various operating conditions.

It is desired to perfect the fitting by the method of least squares. This can be done by generating the spectrum as a periodic function, subtracting from it the sum of distribution functions produced by the computer, and feeding the difference into a square-law meter. We have acquired the meter and are currently developing a function generator which will have better accuracy and stability than the Photoformer presently in use.

The possible further use of the device in analyzing derivative curves, e. g., the commonly used form of presentation in nuclear magnetic resonance experiments, and in integral curves, such as polarographic determinations or titrations will be explored.

See also "Machine Analysis of Absorption Spectra", J. E. Hayes, in this annual report.

Part B included - NO

Serial No. NHI-286
Laboratory of Technical Development
Bethesda 14, Maryland

PHS-NIH
Individual Project Report
Calendar Year, 1958

Part A.

Project Title: Mathematical Investigation of Biological Transport Problems

Principal Investigator: John L. Stephenson

Other Investigators: Marion Sharpless
Arnold Jones

Cooperating Units:

Dr. Donald Fredrickson - LCPM

Man Years (calendar year 1958): Patient Days - None
Total: 1.15
Professional: .4
Other. .75

Project Description:

Progress During Past Year: Began 1957

The general purpose of this research has been to develop a general theory of transport phenomena in linear biological systems. The prototype of the system being studied is that in which a single input $\gamma_a(t)$ is related to the output $\gamma_b(t)$ by an integral equation of the type $\gamma_b(t) = \int_0^t \gamma_a(\omega)\, w(t-\omega)\, d\omega$, where $w(t)$ is a "transport" function characteristic of the system. This type of equation occurs in a variety of physical problems, particularly in the theory of linear electrical filters. It has also cropped up in a variety of biological problems including ageing, circulation problems, and metabolism.

We have analyzed the conditions under which a biological system does possess such a transfer or transport function in detail. The solution of the equation is more or less routine, and we have applied it to simple problems in tracer kinetics in which the input and output are sums of exponentials to derive explicit formulae for "turnover time" and "pool" size which are applicable whether or not the pool is uniformly mixed.

The theory has been extended to systems in which several fluxes of particles are related by a system of such integral

Progress During Past Year(Continued)

equations and appropriate matrix methods for analyzing such systems have been developed. This general theory has been shown to give the well-known solutions for systems which consist of several uniformly mixed compartments. Utilizing the theory general problems of data analysis and model construction in syatems with incomplete information have been analyzed. This work has been written up for publication.

Practically, the theory has been applied to the analysis of tracer data on fatty acid metabolism in collaboration with Dr. Donald Fredrickson. Here, the primary problem was to compare the metabolic pathways of unesterified fatty acid (UFA) and chylomicron triglyceride fatty acids (TGFA). The approach was to assume that flux of UFA back into the plasma pool and carbon dioxide produced by its metabolism would each be related to flux of UFA out of the plasma pool by an integral equation of the above type. All of these fluxes are experimentally measurable; hence the functions $w_1(t)$ and $w_2(t)$ occurring in the integral equations can be determined. In subsequent experiments with TGFA, if these functions remain the same, (which was experimentally tested) they can be used to compute carbon-dioxide production from labelled UFA in the plasma and hence by difference from total excretion of labelled carbon-dioxide, that due to TGFA. Hand computations were carried through according to this scheme using the above theory and estimates of recycling of UFA and the Fraction of TGFA which passes through the plasma pool as UFA before being metabolized obtained. Subsequently, in order to make practical comparison of transport functions in groups of experimental animals or in clinical studies, the computation was programmed for the IBM by Mrs. Sharpless.

Recently, comparison of UFA recycling in fasting and exercising subjects has raised doubt about one of the fundamental assumptions, namely, that all UFA in the plasma pool has the same turnover time. This is now being checked experimentally.

Direction of Current Research:

The immediate program is to apply the general theory to particular problems in biological systems, such as the application to fatty acid metabolism described above.

Part B included - No