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Report of progress activities

Summary of Intramural Research Activities

1957

Laboratory of Cellular Physiology and Metabolism -- Cellular Physiology Section

During the past year the Laboratory of Cellular Physiology has concerned itself with the following major research projects: (1) the structure of ribonuclease and its correlation with biological activity; (2) studies on the mode of involvement of genetic material in protein synthesis, with particular emphasis on the synthesis of bacteriophage protein; (3) studies on the structural role of proline and hydroxyproline in proteins, and on the physical properties of proteins in relation to their amino acid sequence and secondary and tertiary structure; (4) mechanisms of protein biosynthesis in the hen oviduct; (5) structure and metabolism of mucopolysaccharides; (6) structure and function of lipoproteins; and (7) energy transfer associated with electron transport.

(1) The studies relating details of structure of ribonuclease to its catalytic activity have been continued in the direction of chemical removal of larger and larger portions of the native molecule without affecting function. Outstanding among the findings of the past year has been the observation that one, and perhaps two, of the four disulfide bridges in the protein can be opened by reductive cleavage, using sulfhydryl compounds as reagents, without inactivation. The exact location in the three-dimensional fabric of the protein of these two noncatalytic bonds is now under consideration. A series of studies has also been carried out which indicates a close correlation between certain of the spectral and optical rotatory properties of this protein with activity. The enzyme has been isolated in pure form from sheep pancreas and this material has been compared with the bovine protein for differences in sequence and amino acid composition. The significant differences which do exist between these proteins appear to be located within that region of the molecule which other studies have indicated to be indispensable. It is planned to investigate ribonucleases from other biological sources and to examine further degradations in an attempt to reach an active fragment of minimum size.

(2) Due to the advances in genetic techniques, various laboratories have been successful in mapping the genetic material of bacteriophages with a high degree of resolution. One of the mapped regions

controls the biosynthesis of a protein in the tail of the phage which determines the host specificity. The isolation of this particular protein is well under way and techniques have been developed for the comparison of samples of this protein isolated from various mutants, in regard to amino acid sequence. The problem has basic importance in connection with the question of whether or not genetic information is transferred in a 1:1 fashion from DNA to protein during protein biosynthesis.

(3) Studies have been made of the three-dimensional structure of polyproline and various collagens as one approach to the determination of the relative importance of proline as a specific configuration-conferring residue in polypeptide chains. A variety of physical techniques supports the concept of an isomerization about those points in polypeptide chains which contain proline. In other physical studies of globular proteins, considerable information has been obtained on the conditions under which polypeptides become coiled into helical structures. It appears likely that such coiling is favored by conditions where the activity of water is greatly reduced, such as the addition of high levels of lithium bromide.

(4) A fractionation of the protein synthesizing systems of the hen oviduct has indicated the presence of a particulate fraction associated with the "cell debris" of the tissue which may be the site of temporary accumulation of activated amino acid intermediates. Attempts to identify the chemical nature of these intermediates are in progress. There seems little doubt that protein synthesis in this specialized tissue involves precursors other than free amino acids themselves.

(5) As an outgrowth of earlier studies on the degradation of heparin and other compounds by bacterial enzymes, a number of enzyme preparations have been isolated from bacteria which can cause the specific degradation of various polysaccharides at chemically identifiable bonds. The list of enzymes includes at present a hyaluronidase type, a chondroitin type, a sulfatase, a sulfamidase and others with less defined characteristics. In collaboration with Dr. Karl Meyer of Columbia University, degradation products of heparins and other polysaccharides are being separated and investigated. The sub-units of structure should be of great value in subsequent studies on the biosynthesis of polysaccharide molecules. Investigations are also under way to examine the value of mast cell tumors as material for the study of heparin biosynthesis, employing as precursors the sub-units prepared by enzymatic degradation as described above.

(6) A direct chemical study of various lipoprotein fractions of plasma, including chylomicrons, indicates that there are a number of distinct and separable protein moieties which are associated with different density classes of lipoproteins. Most of the work along these lines has so far involved amino acid and group analysis. Recently a technique of "fingerprinting" has made it possible to demonstrate that chylomicrons contain a protein identical with the protein moiety of β-lipoprotein. This technique, depending on the two-dimensional paper chromatographic analysis of peptide mixtures resulting from the proteolytic digestion of proteins, promises to be of general use in the further comparison of other lipoprotein fractions.

(7) Using techniques of ultrasonic disintegration, a subparticulate preparation has been made from isolated mitochondria which is extremely efficient in the process of oxidative phosphorylation. A study of this process in these active preparations suggests that the formation of high-energy phosphate bonds in tissues during the oxidation of substrates may take place by a reversal of the reactions which lead to ATP degradation. An essential step indicated by these studies in the formation of high-energy phosphate bonds involves the production of a high-energy form of a critical enzyme whose nature is at present completely obscure. Present efforts are directed towards attempts to obtain independent evidence for the suggested relation between the phosphorylation process and ATPase activity. The crude system is also being studied in regard to the ability of thyroxin or triiodothyronine to modify the energy-yielding reactions.

Laboratory of Cellular Physiology and Metabolism -- Enzymes Section

The activities of this group are concerned mainly with studies at the enzyme level of various problems of intermediary metabolism. In view of the fact that activated (energy-rich) derivatives of simple metabolites are vehicles for the biological transfer of chemical energy, such compounds are common intermediates in many diverse metabolic pathways and may therefore be regarded as strategic centers in the regulation of metabolism. Accordingly, attention has been directed to a study of selected biochemical systems which offer opportunities to investigate the exact nature of various kinds of activated intermediates and the types of reactions that they undergo. In view of the now well-established fact that the enzymatic mechanisms of energy transfer in metabolism are virtually the same for all living organisms, the experimental material offering the greatest technical advantage has been chosen irrespective of its natural

origin. Specifically, the current investigations are concerned with studies on the metabolism of one, two, and three carbon compounds; the metabolism of amino acids; the metabolism of heterocyclic, ureido and guanido compounds; the metabolism of steroids and other isoprenoid derivatives; electron transport mechanisms; the biochemistry of protein synthesis, cellulose synthesis and differentiation; and the chemistry of purine and pyrimidine basis and their nucleotide and nucleic acid derivatives.

Further studies have been carried out on the enzymatic synthesis of acetyl imidazole by reaction of acetyl CoA with imidazole. This reaction represents a new type of acyl-transfer at the energy-rich level. The extraordinarily high free energy of hydrolysis of acetyl imidazole (ca. 13,000 calories) suggests that it or related derivatives may have an important role in acyl-transfer reactions. The enzyme catalyzing its formation has been partially purified and the kinetics and substrate specificity of the enzyme have been investigated.

Evidence has been accumulated from several sources indicating a central role of three carbon compounds in the biosynthesis of antibiotics and in the oxidation of fatty acids with odd numbers of carbon atoms. In studies on the mechanism of propionic acid oxidation, propionyl CoA, acrylyl CoA, β-alanyl CoA, lactyl CoA and β-hydroxy propionyl CoA have been identified as energy-rich 3-carbon metabolites. The enzymes catalyzing the interconversions of these substances, and the further metabolism of these compounds are still under investigation.

The role and nature of activated one-carbon compound in metabolism is being studied from several points of view. Implication of tetrahydro folic acid as a mediator of one-carbon transfers in the metabolism of purines and in enzymatic conversion of serine to glycine has prompted a more detailed study of the latter reaction. As presented in previous summaries of this research, it was found that numerous folic acid derivatives containing three to six glutamyl-residues, as well as various nucleotide derivatives serve as more reactive coenzyme forms of folic acid in serine degradation. The previously reported unidentified folic acid intermediate in the serine to glycine conversion has now been shown to be dihydro-teropterin. Enzymatic reduction of teropterin to dihydroteropterin is coupled with the oxidation of pyruvate to acetyl CoA and CO_2. This rather specific reaction provides a mechanism by which pteridines are reduced to a form that is biologically active in one-carbon transfer, and it also focuses attention on the possible role of pteridines in electron transport.

In search for other biochemical systems that are particularly well-suited for studies of one-carbon metabolism, the enrichment culture technique has been used to isolate bacteria that can grow anaerobically on simple compounds such as methyl urea, dimethyl urea, betaine, choline, dimethyl glycine, etc. Anaerobic dissimulation of such compounds would be expected to yield products involving the condensation of two or more one-carbon derivatives. Preliminary results showing the conversion of betaine to sarcosine, acetate and ethanol and of choline to trimethyl amine, ethanol and acetate are particularly encouraging. A detailed enzymatic analysis of these fermentations is now in progress.

Studies on the microbial degradation of riboflavin and its analogues were continued as part of a general program designed to determine more about the intermediary metabolism of heterocyclic compounds. Several compounds representing various stages of riboflavin degradation have been isolated in significant quantities as crystalline products of metabolism. One of these has been identified as 3, 4 dimethyl-δ-pyrone and is presumably derived from the xylene moiety of riboflavin. Identification of the other products is in progress. To facilitate further studies in the biochemistry of heterocyclic compounds, microorganisms have been isolated that have specialized abilities to obtain all their energy, carbon and nitrogen for growth through the dissimilation of individual drugs, alkaloids and vitamins. Detailed enzyme studies on the metabolism of some of these is anticipated during the forthcoming year.

Since saw fly larvae (Neodiprion pratti pratti) live exclusively on a diet high in terpenes (pine needles) enzyme preparations of this insect have been examined for their abilities to metabolize various isoprenoid derivatives such as cholesterol, β-ionone, vitamin K and δ-tocopherol. Preliminary results indicate that these are all actively metabolized by extracts of acetone powders. Several enzymatic products of cholesterol degradation have been isolated and are being characterized. A further insight into the mechanism of isoprene metabolism is expected from studies recently initiated on the metabolism of microorganisms that can utilize simple isoprene derivatives (i.e., citronellol, geraniol, camphoric acid, β-ionone, carotene, vitamin K) as sole sources of carbon and energy.

Continued efforts to elucidate the mechanism of ATP formation in the reductive deamination of glycine to acetate and ammonia have led

to a separation of two enzyme components both of which are needed for the overall reaction. When mixed in appropriate proportions these enzyme fractions catalyze on esterification of orthophosphate in excess of the acetate produced with a concomitant accumulation of an unidentified volatile glycine degradation product.

Two enzymes were found to be required for the reductive cleavage of L-proline to form δ-amino valeric acid (1) a racemase, catalyzing the conversion of L to D-proline and (2) a D-specific proline reductase.

An organism catalyzing the anaerobic fermentation of 2 moles of δ-amino valeric acid to one mole each of propionic, acetic and valeric acids and 2 moles of ammonia was isolated by the enrichment technique and it should provide an excellent medium for further studies on reduction deamination.

By analogy to the metabolism of citrulline and arginine, it has appeared likely that the degradation of creatine and creatinine would lead to the intermediary formation of carbamyl-phosphate or a similar energy-rich derivative. Accordingly, two anaerobic bacteria have been isolated that utilize creatinine as major carbon, nitrogen and energy sources. One of these organisms catalyzes the almost quantitative conversion of creatinine to the ureido derivative 5-methyl hydantoin and ammonia. This unique hydrolysis reaction appears to be associated with the formation of one mole of ATP. The other bacterium catalyzes the conversion of creatinine to sarcosine, ammonia and CO_2. Further enzyme studies on the mechanism of the energy-yielding reactions in these fermentations is under investigation.

Recent advances in the metabolism of fatty acids and amino acids have invalidated the common conclusion that flavin enzyme systems are involved only in the transfer of electrons from substrates or pyridine nucleotides to terminal electron acceptors, i.e., cytochromes or oxygen. To obtain more information on the role of flavin enzyme systems as electron carriers in biosynthetic reactions, the mechanism of electron transport in anaerobic bacteria (where terminal respiration involving molecular oxygen or cytochromes is not possible) is being studied. The latter organisms offer a unique opportunity to investigate oxidative phosphorylation at the flavin level uncomplicated by terminal respiration.

Another study dealing with electron transport is concerned with the mechanism of hydrogen activation by a hydrogenase system found in Clostridium kluyveri. The reduction of DPN by molecular hydrogen requires the presence of a heat stable co-enzyme found in extracts of a variety of organisms. The isolation and characterization of this co-enzyme is in progress.

During the life cycle of the slime mold, aggregation of individual myxamoebae to form a migrating slug is stimulated by the presence of a diffusable, chemotactic substance elaborated by the amoebae. It has been found that a similar chemotactic response is obtained with estrane sulfate (0.01 /ml.) and various other steroid hormones. Following development of the slug, further differentiation is manifested by the production of a visible cellulose stalk on top of which sits a spore sac. The overall process of differentiation occurs very rapidly (12 hours) and is associated with a change from a more or less anaerobic to aerobic metabolism. It has been found that coincident with the changes in metabolism there is a marked decrease in the total protein content and in the enzyme composition of the organism. Large increases occur in the concentration of isocitric dehydrogenase, malic dehydrogenase and other enzymes of the TCA cycle. In addition UDPG pyrophosphorylase increases markedly suggesting a role of this enzyme in cellulose synthesis. In view of the rapidity of the differentiation process and the marked changes in metabolism associated with it, and the further fact that these changes occur in the absence of an external food supply, this system offers unique opportunities to study the biochemistry of protein metabolites, cellulose synthesis and differentiation.

In addition to the enzyme studies, research on the chemistry of purine and prymidine bases, their nucleotide and nucleic acid derivatives is also being carried out. Application of infra-red absorption studies to these compounds in D_2O solution has shown that the major tautomeric form existing in solution is the keto form for the uridine and thymidine derivatives and the amino form for adenine derivatives. Extension of these studies to homologous nucleic acid polymers and co-polymers of adenine and uridine nucleotides reveals similar tautomeric forms in the latter compounds as well. The results are consistent with the Watson-Crick hypothesis for a double helical structure for DNA stabilized by hydrogen bonding between the amino group of the purine and the keto group of the pyrimidine bases respectively.

Laboratory of Cellular Physiology and Metabolism -- Metabolism Section

The research of the Section on Metabolism over the past year falls into several categories:

(1) Lipid transport and metabolism and the relation of lipid metabolism to atherosclerosis; (2) Studies of factors controlling serum lipoprotein levels and of factors controlling lipoprotein production; (3) Mechanisms of protein synthesis and degradation; (4) Studies of serum proteins and their metabolism; (5) Studies on experimental and clinical nephrosis; (6) Studies on the mechanism of action of Orinase; (7) Studies on the structure of skeletal muscle proteins.

(1) Further studies on the unesterified fatty acid (UFA) fraction plasma in patients have shown that in the fasting state this fraction is apparently the sole source of respiratory CO_2. The specific radioactivity of the respiratory CO_2 was found to be very nearly the same as that of the UFA following an intravenous injection of the latter. Parallel evidence for the key position of this fraction has been obtained in studies on diabetics, in which case the specific radioactivity of urinary ketone bodies is found again to be the same as that expected if they arose exclusively from plasma UFA. In vitro studies of adipose tissue show that epinephrine stimulates the release and glucose inhibits the release of fatty acids from depot fat. Tissues taken from fasting animals release fatty acid more rapidly than tissues taken from fed animals.

These findings taken together lead to this picture: in the fasting state the unavailability of glucose causes release of fatty acids from the depots. These fatty acids are transported to the sites of combustion in the form of unesterified fatty acids bound to albumin. That this is the only important transport form for getting depot fat to the sites of oxidation is shown by the labelling studies discussed above.

The removal of chylomicron triglyceride from plasma does not take place exclusively through intravascular lipolysis. The body distribution of labeled fatty acids is very different when they are given as UFA and when they are given as chylomicron triglycerides. Apparently the chylomicron fat reaches sites of oxidation without being necessarily transported through the serum as UFA. Furthermore, the clearing of chylomicra is not inhibited by protamine, although the oxidation of triglycerides

in chylomicra is inhibited. Apparently triglycerides can be removed intact from postprandial serum.

It has been shown that the specific radioactivity of the chylomicron protein after feeding labeled amino acids is highest in the first hour and then declines rapidly. This is in contrast to the results generally obtained for serum proteins and suggests the possibility that the protein of the chylomicron is synthesized within the gut wall.

In cooperation with the Technical Development Laboratory attempts are being made to develop an improved sensing device for use in vapor phase chromatography.

Extensive studies on the physico-chemical properties of the serum albumin-UFA complex have been completed resulting in the establishment of association constants for six of the commonly encountered long-chain fatty acids.

(2) In the course of studies of inhibition of cholesterol biosynthesis by Δ^4-cholestenone it has been found that this material profoundly suppresses the production of adrenal steroid hormones. This unexpected but highly interesting finding may indicate that cholesterol is after all 'essential' as a precursor of adrenal steroids. Other possible mechanisms have not yet been ruled out. In collaboration with Dr. Ralph Peterson of NIAMD, it has been shown that the adrenal steroid output of the rat can be decreased to less than one-thirtieth of the control rate expressed as micrograms per gram of adrenal gland per minute, or to about one-sixth of the control when expressed as micrograms per minute.

Further studies with animals and with patients have shown that Δ^4-cholestenone is rapidly absorbed and rapidly converted to dihydrocholesterol which, at high dose levels, accumulates in the liver, the serum and the adrenal gland. In patients the accumulation in the serum was particularly striking even at doses causing little accumulation in animals. This, together with the elevation of BSP retention observed in some patients, rules the drug out as a therapeutic agent. However, in view of the promising results in respect to adrenal function and the profound inhibition of synthesis caused by Δ^4-cholestenone related structures are being investigated.

Further evidence of the distinct difference between the proteins of the α_1- and the β_1-lipoprotein has been obtained by demonstrating the

strikingly different patterns of peptides resulting from proteolytic breakdown. Serum albumin was treated in the same way since it has the same N-terminal group as does the β1-lipoprotein but a distinctly different pattern was observed. The effects of different dietary fats on lipid metabolism have been investigated. It has been shown that unsaturated fats do not, as was postulated, act by inhibiting cholesterol biosynthesis. Rats fed large amounts of corn oil in the diet actually showed greater rates of cholesterol synthesis than rats fed coconut oil, a highly saturated fat. A series of patients is being studied on liquid formula diets containing corn oil or coconut oil. Bile acid and sterol excretion in the feces is being measured. The patients have received labeled cholesterol prepared by tritiation according to Wilzbach, which proves to be a highly useful and economical way of obtaining compounds. An unexpected and important finding is that the fecal sterol excretion actually exceeds by several fold the bile acid excretion. Apparently the pattern in man is quite different from that in rats. This may help to account for the very different reaction of the two species to dietary fats. Rats receiving even 20% corn oil by weight in their diets show only a very small percentage fall in serum cholesterol levels whereas our patients have shown drops of 30 and 40%.

Patients with nephrosis and very low serum albumin concentration have long been recognized to have fairly consistent hyperlipemia. It has been shown previously that treatment with steroids results in a fall of the serum cholesterol levels simultaneous with the rise in serum albumin level. It has now been shown that elevation of the serum albumin level by infusion of albumin likewise returns the lipid levels towards normal. The mechanism by which this is accomplished is under study.

(3) The limits of the specificity of the protein synthetic mechanism have been frequently discussed. Information on this point is relevant in arriving at a picture of the mechanism of synthesis. Using tritium labeled amino acid analogues and a system of radioassay developed in this laboratory it has been shown that amino acid analogues can be incorporated into well defined crystalline proteins. This result eliminates an objection frequently raised to analogue incorporation experiments in which unfractionated mixtures have been studied since these might contain partially completed protein molecules containing the analogue but no intact protein structure. The analogues studied (o-fluoro-phenylalanine and p-fluoro-phenylalanine) are structurally very closely related to the normal amino acids. In fact, the analogues are more closely related to their normal counterpart than are homologous amino acids in

nature related to each other. Further studies with aromatic amino acid analogues are planned to probe the limits of the specificity of the synthetic mechanism.

Further evidence for the presence of bound amino acids in liver has been obtained and some suggestive evidence that these amino acid complexes may be intimately connected with nucleotides.

A method for preparing tritium-labeled proteins by the Wilzbach procedure has been worked out, as well as a system for radioassay of intact proteins in the liquid scintillation spectrophotometer.

(4) The hypoproteinemia frequently observed in patients with regional enteritis or ulcerative colitis has been shown to be due most probably to a steady loss of serum protein into the intestine through the damaged areas. Using polyvinyl pyrrolidone (PVP) as an indicator it has been shown that patients with defective intestinal mucosa lose this material rapidly into the lumen of the intestine.

A method for measuring trypsin activity in blood has been developed. The kinetics of the system are rather unusual because of the presence of a potent inhibitor in normal serum. Nevertheless it appears to be possible to measure trypsin added to normal plasma or serum by the use of the specific synthetic substrate in a simple straightforward procedure.

Attempts are being made to produce serum albumin with double molecular weight by making a dimer through a disulphide bridge. If the product is retained by the nephrotic glomerulus it might be of use in therapy. In addition it would permit restoration of normal serum albumin levels to permit studies of fatty acid metabolism in these patients

(5) Studies on the treatment of nephrosis with steroid hormones continue to demonstrate that excellent remission rates can be obtained. Eleven out of 19 children had complete remissions and 16 had at least partial remission.

Because of the reported effectiveness of chloroquine in lupus erythematosis a trial of this drug in nephrosis was instituted. Of 6 patients receiving chloroquine only one had a remission and 4 who failed to respond to chloroquine responded well to subsequent steroid therapy.

The relationship between serum albumin levels and the rate of protein loss in the urine has been studied in a large number of patients. Although final conclusions cannot be drawn, the data set limits on the extent to which rate of synthesis and rate of degradation may be involved in leading to the abnormally low serum albumin levels. Laboratory studies directed at identifying the protein antigen responsible for production of nephrotoxic serum continues. The material has been somewhat purified but work has been hindered by the tendency of the material to spread over many fractions both in zone electrophoresis and in alcohol fractionation.

Attempts to demonstrate antibodies against human kidney in the serum of 30 patients with renal disease were negative. On the other hand, several patients with lupus erythematosis have been found to have such antibodies in their serum and this is being further studied.

(6) Further studies on the mechanism of action of Orinase were directed at determining whether liver phosphorylase was involved. When Orinase prevents the effect of epinephrine on liver slices in vitro it can be shown that it is also preventing the increase in active phosphorylase that normally follows the exhibition of epinephrine or of glucagon.

(7) The ribonucleic acid found in myosin preparations has been separated from it by the use of cellulose ion exchange columns. The purified nucleic acid has been analyzed for its base composition. It was of great interest that this composition remained constant from preparation to preparation and despite wide variations in the extent of fractionation to which the RNA samples were subjected. Studies in the ultracentrifuge demonstrated the heterogeneity of these materials but in view of the constant composition in different samples this may represent different size molecular aggregates of the same basic molecule. Some information about the nature of the binding of the RNA and of its configuration was obtained by spectral studies. Further studies of physical properties of myosin from cardiac muscle are planned.

Ultracentrifugal properties of heart muscle myosin in normal dogs and dogs with experimental cardiac failure were carried out in cooperation with Dr. James Davis.

Laboratory of Chemical Pharmacology

Development of New Drugs

Antirheumatic Drugs: Butazolidin is of value in treatment of arthritic diseases. It does not exert hormonal actions, but it causes a number of other side effects. A compound like Butazolidin but lacking its side effects would be of considerable clinical importance. A search for such a drug is being carried out in collaboration with Mt. Sinai and Goldwater Memorial Hospitals, New York, and Geigy Pharmaceutical Co., Basel.

To date, 55 Butazolidin analogues have been studied, and structural features required for various actions have been outlined. Substitution in the para position of the benzene ring yields compounds with marked antirheumatic and sodium retaining effects; substitution in the ortho position also produces compounds causing marked sodium retention; substitution in the meta position, however, yields compounds that do not produce sodium retention but still have some antirheumatic activity. Any change in butyl sidechain results in potent uricosuric agents having little or no antirheumatic and sodium retaining properties. In general a change which increases the acidity of the molecule increases its uricosuric action.

These observations on relationship between chemical structure and pharmacological activity are aiding in the development of more useful drugs. Thus far, two new drugs have been discovered and are now under extensive clinical trials. One drug, a para hydroxyl metabolic product of Butazolidin (Metabolite I), has potent antirheumatic activity in acute gout and rheumatoid arthritis. It is proving to be effective in many patients who fail to respond to Butazolidin. The other, G-28, is the most potent uricosuric agent yet described and appears to be of considerable value for the treatment of chronic gout. In addition compounds are now at hand which are potent antirheumatic and uricosuric agents.

Muscular Relaxants: There is need for an effective muscular relaxant for treatment of diseases associated with peripheral muscular spasm. Recently, Flexin has been introduced as a drug which acts centrally. Our studies have shown that Flexin is metabolized by substitution of a hydroxyl for the amino group, to a compound which also has muscular relaxant properties, but has the advantage over the parent drug in being rapidly and completely absorbed, and in being less toxic. Extensive clinical studies indicate that the metabolite is an effective drug and it will probably replace Flexin.

Biochemistry of Function

A main theme of the Laboratory is that the specialized functions of various organs can be explained biochemically only in terms of reactions that are unique for each organ and not in terms of the universal enzyme systems present in most living cells. It may be presumed that therapeutic agents exert their effects by modulating these organ-specific enzyme systems.

Biochemistry of Brain Function: We have found that the long neglected concepts of W. R. Hess are invaluable as reference points in bridging biochemical, anatomical, physiological and pharmacological aspects of brain function. Hess showed that two opposing systems (Ergotropic and Trophotropic) integrate autonomic, somatomotor and psychic functions in brain stem. Activation of the ergotropic system yields central effects like those caused by LSD and mescaline; while activation of the trophotropic system elicits responses similar to the effects of reserpine and chlorpromazine (see table).

Hess regarded the two systems as constantly active but in a state of equilibrium. We have considered that the two opposing systems require different synaptic transmitting agents and that reserpine and LSD upset the equilibrium influencing the relative excitability of either system by affecting synaptic transmission. We believe that the transmitting agents are serotonin and norepinephrine. These substances satisfy certain minimal criteria for the role of transmitters: 1) They are formed in central nervous system, are present in a stored form and are concentrated in those parts of the brain in which centers described by Hess are present, 2) the enzyme that inactivates both amines is present in brain and is especially concentrated in the hypothalamus, 3) introduction of small amounts of serotonin and norepinephrine into brain causes marked central effects, 4) blocking the enzyme that inactivates the two hormones causes a marked central effect, and 5) compounds that block the action of serotonin or of norepinephrine in brain exert marked central actions.

Evidence that norepinephrine is the neurohormone of the ergotropic system is now fairly definite. There are many indications that serotonin has the counterpart role in the trophotropic system, but there is still conflicting evidence.

Classification of Drugs: A number of drugs may be classified in terms of their interaction with the trophotropic (serotonin) and the ergotropic (norepinephrine) systems. Reserpine activates the trophotropic system. It acts indirectly by impairing the serotonin binding sites; as a result the serotonin that still forms is freely available to the synapses of the trophotropic system. Chlorpromazine has been shown to induce similar central effects by acting as a central noradrenergic blocking agent, thereby blocking the ergotropic system. Drugs like mescaline, amphetamine and ephedrine activate the ergotropic system by simulating the action of norepinephrine. Other drugs like bufotenine and perhaps LSD may act by blocking the trophotropic system by interfering with the action of serotonin.

The action of reserpine is complicated since it also impairs the capacity of brain cells to store norepinephrine. Consequently, the drug exerts mixed effects, with trophotropic stimulation predominant. The peripheral stores of norepinephrine are extremely sensitive to reserpine action and are released by very small doses of the drug. Consequently the peripheral autonomic effects of reserpine are due not only to increased parasympathetic activity, but to the inability of the sympathetic system to respond to stimulation.

Certain synthetic benzoquinolizine derivatives exert reserpine-like effects though they are not indoles. These drugs release brain serotonin and norepinephrine but unlike reserpine they are short acting and appear to exert effects only while they are present in the body. In contrast, reserpine acts long after it has disappeared. When the new compounds are given to rabbits before reserpine the long lasting effects of the Rauwolfia alkaloid are inhibited. This indicates that similar receptor sites are involved in the action of both types of drugs. Of great interest are the indications that the new compounds do not liberate serotonin or norepinephrine from peripheral depots. If verified this will be important in defining the central role of the two hormones.

Although ergotropic agents induce effects opposite to those of reserpine, animals pretreated with reserpine react more strongly to the ergotropic actions of amphetamine, cocaine, etc., than do control animals. The nature of this potentiation is not clear, but is reminiscent of the potentiation of epinephrine after sympathetic denervation. LSD action is also potentiated suggesting that it may not act, as commonly thought, as a serotonin antagonist in brain, but by simulating norepinephrine

On the other hand, 5-hydroxytryptophan does not reverse the effects of reserpine indicating that the stimulatory effects of this substance are due to a different mechanism. Preliminary results show that 5HTP in addition to increasing the level of brain serotonin, also releases norepinephrine.

Inhibition of Monoamine Oxidase: A controversial question is whether catechol amines are metabolized by action of monoamine oxidase or by enzymes that yield adrenochrome. We have demonstrated that both serotonin and norepinephrine, released in brain, are inactivated by monoamine oxidase only. It may be concluded that the predominant pathway of norepinephrine metabolism in brain is oxidative deamination and that the extent of oxidation to the "hallucinogenic" adrenochrome is negligible.

Marsilid which blocks the action of monoamine oxidase induces a rise in the brain level of both serotonin and norepinephrine. Studies with rabbits have shown a relationship between the rise in level of brain amines and the onset of stimulatory effects. This supplies a clue as to the action of the anti-depressant action of Marsilid in treatment of mental patients. Its effects in relief of pain and in rheumatoid arthritis may also be related to the rise in brain norepinephrine which has been reported to exert an analgesic action.

JB-516 (α-methyl, β-phenyl-ethylhydrazine) has been shown to be an extremely potent monoamine oxidase inhibitor in vivo. It also induces a rise in brain levels of serotonin and norepinephrine and produces central stimulatory effects with very small doses of drug.

Neither of the monoamine oxidase inhibitors changes the level of the amines in peripheral organs, presumably since the storage depots are already saturated. However, they do cause a marked rise in blood serotonin.

Since the pharmacologic effects of Marsilid are evident only when the brain levels of the amines reach a plateau, it does not appear that the inhibitor bears the same relationship to the two hormones that physostigmine does to acetylcholine. The indications are that the function of monoamine oxidase in brain may be to regulate the amount of monoamines in storage depots rather than to inactivate them after their release.

Miscellaneous: Norepinephrine has been definitely identified in rabbit and dog heart in high concentration, about 2 γ/gm. The level in atrium is about twice that in ventricle but otherwise the substance is fairly uniformly distributed in heart tissue. Attempts are being made to ascertain whether the hormone has any function in heart other than that of a sympathetic mediator.

In collaboration with the Laboratory of Kidney and Electrolyte Metabolism, studies on digitalis-like material in tissues have been continued. The material isolated from adrenal glands has been identified as β-palmitoyl lysolecithin. It is present mainly as an inactive form containing a long chain fatty acid bound to the inactive lysolecithin by a labile hemiacetal. Similar substances are present in heart and in plasma. Ether-soluble digitalis-like substances are also present in heart and serum. The serum substances are the two fatty acid derivatives $\Delta^{3,4}$ cis octadecanoic acid and $\Delta^{11,12}$ cis octadecanoic acid. The substance in heart appears to be different. Attempts are being made to implicate these digitalis-like substances in the functioning of cell membranes.

A substance which fluoresces at 460 mμ when activated at 360 mμ is present in a number of organs. It has been purified and shown to be a very weak acid which appears to be a mixture of three substances with similar properties. They are apparently unknown compounds and should soon be available in pure form so that their function may be determined.

Membrane Permeability

Foreign Compounds: Studies on the passage of foreign compounds across body membranes (collaboration with Laboratory of Kidney and Electrolyte Metabolism) have been based on the simplifying assumption that cell surfaces are essentially lipid membranes across which foreign compounds penetrate by virtue of their lipid solubility. On this basis the rate of passage of a drug across a membrane should depend on the dissociation constant and on the pH of the absorbing site. The rates at which drugs penetrate gastric mucosa, intestinal mucosa, kidney tubules and blood-brain barrier were found to be in accord with this concept. While much still remains to prove the thesis, the results thus far permit accurate predictions of the absorption, excretion and penetration into brain of a drug from its physicochemical properties.

The work in the past year has been concerned with further elaboration of this important concept. With a special technique, the study of gastric absorption was extended to the human. Most acidic drugs were found to be rapidly absorbed while basic drugs, unless very weakly basic, are poorly absorbed. Thus, the human stomach is an important site of absorption for a number of acidic drugs. Some are absorbed more rapidly than ethanol which heretofore has been considered to be unusual in its rapid absorption from stomach.

The technique for measuring absorption from rat small intestine was altered to permit the comparison of strong bases. The results show that drugs like ephedrine and mecamylamine are absorbed much more rapidly than quarternary ammonium compounds or sulfonic acids; as would be expected according to the "lipoid" concept. The rat colon has been studied and the pattern of drug absorption is similar to that found in small intestine.

Studies on blood-brain barrier are continuing. While the preliminary studies have shown that the passage of drugs into the cerebrospinal fluid is dependent on their pKa and lipid solubility, it is still not clear why drugs like salicylate with a pKa of 3 should penetrate into the brain. The possibility that the pH at the actual boundary is lower than 7 is being considered.

Normally Occurring Substrates: Several studies on the nature of the specialized mechanisms that transport polar substances across cell membranes are being carried out. 1) Using platelets as an in vitro model system considerable evidence now favors the concept that serotonin and norepinephrine are held in cells against a concentration gradient by means of a transport mechanism rather than a chemical binding. Reserpine acts by inhibiting the mechanism and the consequent release of serotonin is due to its passive diffusion from the cell. 2) The view that the boundary between the intestine and plasma is lipoidal permits tentative conclusions as to whether a substance is absorbed passively or by a specialized process. Preliminary results indicate that several nucleosides and nucleotides are readily absorbed despite their low lipid solubility, indicating that specialized mechanisms are involved. It is hoped that these studies will tell us whether a few mechanisms account for the absorption of a host of nutrients and whether the same mechanisms are involved in absorption, kidney secretion and in penetration of

blood-brain barrier. 3) A study of the mechanism of biliary secretion, especially of foreign compounds, has been started. Preliminary results show that large molecules like inulin are secreted in small amounts. This observation is difficult to relate with the present views of the nature of bile secretion (collaboration with the Laboratory of Kidney and Electrolyte Metabolism).

Drug Metabolism

Emphasis has been placed on the normal role of the enzymes that metabolize "foreign" compounds. These can be placed into three categories as follows: Substances metabolized by relatively specific enzymes involved in intermediary metabolism. These are the so-called "anti-metabolites" which are structurally so similar to normally occurring substrates that they may become involved in synthetic reactions. Substances acted on by relatively non-specific enzymes involved in intermediary metabolism. An example is aldehyde dehydrogenase which not only oxidizes the aldehyde intermediates in the metabolism of norepinephrine, serotonin, etc., but have been shown to oxidize aldehyde intermediates of drug metabolism. Alcohol dehydrogenase may constitute another example of this kind of enzyme. Both enzymes have been shown to be involved in the oxidation of alkyl sidechains (barbiturates) to the corresponding carboxylic acid. Substances acted on by extremely non-specific enzymes not involved in intermediary metabolism. Examples of these are the compounds oxidized by enzymes in microsomes along a variety of pathways and perhaps compounds that are conjugated to form glucuronides, ethereal sulfates and mercapturic acids. Characteristic features of these enzymes are their non-specificity, inability to act on polar compounds (normal substrates), inhibition by SKF 525-A, and increased activity induced by certain carcinogens. A further characteristic is the invariable conversion of foreign compounds in vivo to less lipid soluble, more readily excretable metabolites. The resulting product is usually less toxic because it cannot penetrate as easily to a potential site of action, but may be more toxic if a different functional group is produced or unmasked.

Comparative studies indicate that many of the systems that metabolize foreign compounds arose in evolution in response to the need to conserve water. Aquatic animals ingest foreign compounds in food, but surrounded by an excess of water and possessing semi-permeable gills or skins easily disposed of the unwanted substances by

passive diffusion. Accordingly fish and certain amphibia do not possess oxidative mechanisms for drug metabolism. These appear in terrestrial animals including reptiles, birds, mammals, toads and insects which do not dispose of foreign compounds through membranes and cannot excrete the lipid soluble compounds via the kidney. The oxidative systems in toads and in insects which developed independently of those in reptiles, birds and mammals appear to be different mechanisms.

Phenols form glucuronides and ethereal sulfates in frogs, but not in fish. These processes are also present in toads but are absent in tadpoles of frogs and toads. It is not clear why it is necessary for amphibia to be able to conjugate phenols, but it indicates a possible important chemical reaction that started with the emergence of life on land.

Insects (crickets) oxidize drugs very rapidly by mechanisms as yet unknown. A most unusual finding was that crayfish, which are also arthropods, slowly oxidize a number of foreign compounds. This has led to the surprising finding that crayfish gills are not permeable to lipid soluble foreign compounds and therefore these animals have had to develop biotransformation systems in order to excrete the compounds in the kidney. The crayfish type of gill is probably associated with the ability of aquatic arthropods to breathe on land.

Miscellaneous: Another oxidative enzyme system has been found in mammalian microsomes. This system oxidizes sulfur-containing compounds, like chlorpromazine, to the corresponding sulfoxide, and requires TPNH and O_2.

An important biochemical problem is the mechanism of hydroxylation. In previous studies with model systems we have shown that active hydroxyl groups convert ring systems to phenols and oxidatively split ethers. Two more of the oxidative microsomal reactions, dealkylation and sulfoxide formation have been carried out by a model system consisting of methemoglobin and H_2O_2. It is possible that all the oxidative microsomal reactions are primarily hydroxylation reactions in which a hydroxyl donor transfers an active hydroxyl group to an acceptor substrate.

Studies on Ascorbic Acid and a New Pathway of Glucose Metabolism

Studies on the increased synthesis of ascorbic acid induced in rats by many drugs have uncovered the following pathway of glucose metabolism:

D-glucose ⟶ D-glucuronic acid ⟶ L-gulonic acid ⟶ L-xylulose ⟶ Pentose cycle ⟶ CO_2. This pathway also occurs normally, but to a much smaller extent. One of the intermediates, L-gulonic acid, is converted to L-ascorbic acid in the rat and other animals that synthesize the vitamin. This step is missing in man, monkey and guinea pig and explains the need for vitamin C in their diet. L-gulonic acid also serves as a precursor of L-xylulose; thus the excretion of this sugar by patients with essential pentosuria is explained. This stimulatory effect of drugs on this pathway of glucose metabolism does not involve glucuronide formation, but appears to be a previously unreported response on the part of the body to foreign compounds.

D-ascorbic acid can replace certain actions of L-ascorbic acid at equivalent tissue levels in guinea pigs. The D-isomer maintains weight, survival and the production of dentine in scorbutic animals but does not prevent hemorrhagic reactions. These data suggest that L-ascorbic acid exerts two types of action only one of which can be replaced by the D-isomer.

The Effect of Drugs on Fat Disposition

It has been known for many years that a number of drugs including white phosphorus and CCl_4 induce fatty infiltration in various organs. Interestingly enough little has been done in elucidating the mechanism whereby these substances upset normal lipid distribution. We have shown that a dose of 0.5 mg/kg of epinephrine or norepinephrine infused over a period of a few hours makes hearts hypersensitive to epinephrine induced arrhythmias for 2 to 5 days. Fatty infiltration was found in heart and in other tissues. In addition the cholesterol levels rose in plasma. The lipid changes can be blocked by pretreatment with dibenzyline. The implications of these findings may be important, as the data suggest that epinephrine may be involved in some way with lipid distribution. It is of interest that white phosphorus does not induce fatty infiltration in adrenalectomized animals.

These studies will proceed from a number of points of reference: a) whether there is common mechanism in fatty infiltration induced by a number of chemical agents, b) whether the common factor is epinephrine and if this hormone has a normal role in releasing fat from depots, and c) whether this control mechanism is upset in atherosclerosis.

STIMULATION OF DIENCEPHALIC SYSTEMS

(Nomenclature of Hess)

Comparison with Effects of Reserpine and LSD

	Trophotropic stimulation (or effects of reserpine)	Ergotropic stimulation (or effects of LSD, mescaline, etc.)
Behavior	Lack of volition and adynamia Decreased reactivity to sensory stimuli Drowsiness, sleep with active closure of eyelids	Increased initiative and emotional responses, increased reactivity to sensory stimuli, arousal and excitement
Skeletal muscle	Decrease in muscle tone and locomotor activity General atony	Increase in muscle tone and locomotor activity Motor excitement
Respiration	Depression	Activation
Autonomic system	Miosis Lowering of blood pressure Bradycardia Relaxation of nictitating membrane Salivation Hypothermia	Mydriasis Elevation of blood pressure Tachycardia Retraction of nictitating membrane --- Hyperthermia

Laboratory of Chemistry of Natural Products

The work of this laboratory during the past year may be summarized in five categories.

A. Isolation Studies -- Almost every chemical investigation requires isolation work of some kind. For the most part this involves relatively routine, although perhaps difficult, isolation steps that are familiar to all organic chemists and which require no particular mention. However, when biological materials are involved, whether they are of animal, plant or microbiological origin, a desired isolation may become so difficult as to require extensive study, and under these circumstances an isolation study may become in itself a fully developed research project in which structural chemistry and related considerations have little or no part. Several such studies are in progress.

Observations by Dr. S. J. Sarnoff and his colleagues on the vasodilating effect of human urine led to the establishment of a joint project directed to the isolation of the active agent or agents. The human vasodilator responsible for the major part of this effect has been the subject of many previous studies. It has been established in past studies that a macromolecular polypeptide called "kallikrein" or "callikrein" is present in human serum bound to an "inactivator," and that free kallikrein is present in human urine. Rather complex relationships involving the conversion of "kallidinogen" into "kallidin" by the agency of kallikrein have been proposed. It is accepted that kallikrein is a pancreatic product. It is not known whether kallidin and bradykinin are identical, although this has been suggested.

The objective of the chemical study is to develop a method for the isolation of kallikrein (the literature methods are unsatisfactory), and the overall objective is to determine the function of this human vasodilator; in particular the question to be answered is whether this very potent compound is involved in the homeostatic mechanisms involved in the maintenance of normal blood pressure.

In pursuing this problem (Dr. Pierce) it was found that approximately 80-90% of the vasodilator activity was due to a macromolecular fraction. An isolation procedure, involving the novel use of an alumina column as an ion exchange medium, was developed. The conditions for the absorption and desorption of kallikrein have been defined; these fall

within narrow limits of pH and ionic strength. The material obtained in this way has a high order of activity, although it is still a mixture. Further purification through XE-64 resin and cellulose DEAE columns is currently under study.

A small-scale kallikrein isolation method, suitable for use with human urine samples, was also developed. It was combined with a bioassay procedure developed in the Laboratory of Cardiovascular Physiology to measure kallikrein excretion in clinical studies.

Future chemical work is aimed at defining and characterizing kallikrein with the aid of modern techniques, and in comparing kallikrein with the therapeutic agent Padutin (an animal pancreatic product believed to correspond to human kallikrein). The work should also provide a procedure for the isolation of pure human kallikrein for physiological studies.

A second isolation problem (Dr. Fish) is concerned with human urine components of the organic base class. This study was influenced by a number of metabolic observations, but the chief reason for its inception is the fact that every organic base now known to be present in the human body has also been found in human urine, and every compound of this class now known to be a human component, has a potent physiological activity. Adrenaline, noradrenaline, histamine and serotonin are examples. Each of these compounds was found first in a tissue other than urine, but later work in each case resulted in the detection of the compound as a urinary component. An obvious way to determine whether new and important human bases exist, in addition to those now known, is to reverse the process and look for them first in urine and secondly in specialized tissues. The levels are of necessity very low because of the high order of physiological activity of the bases.

Another factor of interest is the report from Page's group that bufotenin is present in human urine. While it does not follow that this indole base has any significance, the fact that it may be a normal human plasma component is an observation that deserves checking.

The technical problems of isolating these materials have not been fully solved, but the results to date may be summarized. Dimethyltryptamine oxide, bufotenin and bufotenin oxide are present. The fact that dimethyltryptamine has not been found previously is probably due to its easy conversion to the oxide. The recognition of this substance is a

consequence of Piptadenia work; it is interesting that the two hallucinogenic agents of the plant are also human components. Tryptamine is another indole base in human urine; this observation is of questionable significance since the base presumably originates in the gastrointestinal tract. (Simple primary bases may well originate from this source, although cellular synthesis in specialized tissues may occur at the same time. For example, plasma and urinary histamine is accepted as being derived from both gastrointestinal action and from histamine sources elsewhere in the body.) An observation which may have some immediate significance is the fact that the serotonin fraction contains two components. One is serotonin. The other component is an indole base with quite similar physical properties. In separate studies it has been found that the second compound is a serotonin metabolite. This unexpected observation warrants thorough investigation; the results are being reviewed and checked.

Other observations which have not yet been evaluated have been made. Approximately 8-10 other compounds are normally present in this base fraction. Further study of these will follow.

A third isolation study is projected. "Kuru disease" of New Guinea is characterized by the development of brain lesions, and it apparently is due to the intake of a dietary or ritual material. Four suspected plants have been sent in for investigation; two of these are used for ritual purposes, and they are reported to cause hallucinations. The brain examinations were conducted at NIH.

B. Biological Reaction Studies -- The principal investigation in this area is that of N-demethylation and N-methylation. Following the discovery of an iron-catalyzed rearrangement reaction of amine oxides, a systematic study of the chemistry and enzymatic reactions of these compounds was initiated. This resulted in the finding of an enzyme-catalyzed rearrangement reaction, indicating that a normal substrate undergoes this reaction. Other studies established the chemical route for oxidative decarboxylation of β-amino acids; this has not yet been observed as an enzymatic reaction, although it is thought to occur also as a cellular process.

Current studies (Dr. Sweeley, Dr. Fish) involve both demethylation and methylation work. A chemical study of nicotine oxide has given new information about the oxidation and rearrangement reaction. The

chemical work will be paralleled by studies of the enzyme-catalyzed reactions occurring in a Pseudomonas strain which is known to metabolize nicotine by a route that clearly involves an amine oxide. The reaction of N-methylation may occur through a reversal of the amine oxide N-methylation route, or by way of S-adenosylmethionine. Several N-methylation possibilities have been examined, and one has been selected for further study. The conditions indicate that the S-adenosylmethionine route was involved in this case, but further study will be necessary before a final conclusion may be drawn. (At the present time there are biological experiments that point very clearly to the existence of two different modes of N-methylation. One requires methionine, and the other does not. Presumably the first occurs by way of S-adenosylmethionine, and the second through an unknown THF derivative. The second mode may follow the amine oxide route in reverse.)

This work is of fundamental importance in establishing the mechanisms and relationships between N-demethylation and N-methylation, and it is part of a continuing project on the chemistry of organic bases. Another reaction brought under study during the year (Miss Zaltzman) was the tetrahydroisoquinoline ring closure reaction. This reaction is a common one in plant chemistry, not now known to exist as a mammalian reaction. Very little was known about o- and p-relationships. These were studied, and it was found that the direction of substitution is controlled by pH. While it would be desirable to extend this study to enzyme systems, there are technical difficulties which may make this impossible.

C. Metabolic Studies -- A novel discovery in recent years is the fact that human metabolism includes reactions of m-hydroxyphenyl compounds. These are m-isomers corresponding to the more usual compounds derived from p-tyrosine and o-tyrosine. The origin of these m-hydroxy compounds is not known, although dietary precursors are involved.

One of the immediate objectives of this human metabolic study (Dr. Sweeley) was to isolate and identify the major metabolite in this group of compounds. This was done; the major component is m-hydroxyphenylhydracrylic acid. The same identification was made earlier by M. D. Armstrong, however, and an exchange of data confirmed Armstrong's work.

Although m-tyrosine is not known to be a normal mammalian component, it and its derivatives act as substrates for a large number of enzymatic transformations. Several of these are under study; in particular, a new amino acid has been isolated as a metabolic product in the dog, and it is desired to know whether this is a consequence of either side-chain hydroxylation or side-chain methylation.

Other metabolic studies are projected if amino acid methylation can be demonstrated.

D. Structural Studies -- Much of the basic work of organic chemistry lies in structural studies. A number of plant products of unusual and unknown structure have been under study in the past year. The most extensive work has been on Amaryllis alkaloids (Dr. Wildman, Dr. Fales, Dr. Crowder). At the start of this work, only a few members of the group were known, and these were assigned incorrect structures. Now more than sixty compounds have been described, and the structures are known for most of these. The solutions to the structural problem were provided largely by three groups, one Canadian, one Japanese, and one U. S. In order to complete the remaining phases of the structural study, Professor Uyeo (Osaka University) is spending several months in NHI to compare Japanese data with results obtained here.

No new significant physiological activities were found in this group of compounds; the morphine-like activity discovered last year has not been found to hold for other than one sub-group of the entire family.

The Lunasia alkaloid problem (Dr. Goodwin) is largely solved as far as essential structural features are concerned. The details of the problem are being pursued here and in Australia by Dr. J. R. Price (CSIRO). Several exchanges of data with the Australians were helpful in blocking out the significant relationships. The Ocotea problem (Dr. Goodwin) is also largely solved, although one structural point remains in doubt.

The Ormosia problem (Dr. Lloyd) has not yielded a structural answer, although much information has been obtained. Currently a comparison is awaited with a newly-isolated material described by Galinovsky (University of Vienna).

The Tecoma problem (Dr. Fales) and the Cassia problem (Dr. Highet) are both in progress. Tecoma compounds are under investigation as anti-diabetic agents; this has been their use.

E. Methodology -- Several studies in chemical methodology are in progress. One is concerned with applications of rotatory dispersion measurements. Appropriate equipment has been set up for use by this and other laboratories for investigations in this area.

A second project is concerned with gas chromatographic problems. New liquid phases for high temperature fatty acid ester separations are needed, and several have been prepared for study. The chief difficulty in this work is the lack of suitable equipment.

Laboratory of Kidney and Electrolyte Metabolism

Studies in the Laboratory of Kidney and Electrolyte Metabolism continue to be concerned with the mechanisms by which the quantity and distribution of fluid and electrolytes in the body are regulated. These investigations fall into three major groups: 1) studies of renal processes involved in the excretion of water and electrolyte; 2) studies of the mechanisms involved in the movement of ions and water across biological membranes; 3) investigations of the disturbed physiology associated with experimental heart failure and experimental edema produced by other lesions.

1. Studies of renal mechanisms: A major area of interest and experimental study has been that concerned with the renal processes involved in dilution and concentration of the urine, the final steps involved in osmoregulation. The studies of Drs. Berliner and Davidson completed last year had shown that antidiuretic hormone (ADH) was not essential for the operation of the concentrating process. The observations were fully compatible with the hypothesis that the effect of ADH is entirely upon the permeability of the renal tubules to water. Attention was directed to the nature of the process by which the urine is made hypertonic. As a consequence a hypothesis has been developed which is the first to explain all the known phenomena of the concentrating process and studies (by Drs. Levinsky, Davidson and Berliner, with assistance from Dr. Eden (Laboratory of Technical Development) on the theoretical aspects) have been devoted to its experimental examination. The essentials of

the hypothesis are: 1) that the loop of Henle, like the remainder of the tubule, transports sodium salts from lumen to interstitial fluid, 2) that the loop is impermeable to water so that the urine becomes diluted in its passage through the loop, 3) that the excess of water created by dilution in the loop escapes from the distal convoluted tubule in the cortex, a region anatomically segregated from the loops and collecting system, 4) the urine returning to the medulla in the collecting system loses water to the sodium derived from the loops and becomes hypertonic, 5) the sodium concentration in the medulla is able to rise to high levels because the countercurrent flow of its capillary blood supply yields a marked reduction of the effective blood flow for the removal (or entry) of diffusible substances, 6) the countercurrent trap permits the accumulation of urea in the renal interstitial space after its diffusional escape from the collecting system so that urinary urea can be largely balanced by renal interstitial urea. In accord with this hypothesis the sodium and chloride concentrations of the total water of renal medulla have been shown to be of the order of 350 and 325 mEq/L respectively in the dehydrated dog. The amount of these ions present has been found to be substantially similar in water diuresis in conformity with the view that the operation of the mechanism is not dependent upon ADH. Minor degrees of reduction of filtration rate yield increases in the concentration of the urine produced (control hypertonic) as would result from the smaller delivery of water to the area from the urine. Greater degrees of reduction lead to sharp drops in the urine concentration due 1) to diminished availability of urea to the region and 2) probably to diminished availability of sodium for transport in the loop. These and other studies aimed at exploitation of this hypothesis are continuing.

Drs. Orloff, Wagner and Davidson have completed their studies of the effect of various doses of ADH at varying rates of solute excretion. These have shown that amounts of pitressin adequate to maintain urine hypertonicity when solute excretion is low yield dilute urine when solute excretion is increased. It has been observed repeatedly that at very high rates of solute administration the urine becomes dilute no matter how high the rate of ADH administration. This phenomenon, which is as yet unexplained, is being investigated further.

Drs. Levinsky and Berliner are continuing studies of another unexplained and possibly related phenomenon. It has been repeatedly observed that dogs given continuous dosage of pitressin-tannate in oil

and standard daily water loads exhibit the initial water retention, dilution and sodium loss which has been described before, but that within a week to ten days come back into balance and undergo no further dilution because of a markedly decreased capacity to produce concentrated urine. If put on large doses of desoxycorticosterone, the failure to produce concentrated urine is present from the start and virtually no dilution of the body fluids occurs.

Studies of the mechanism of potassium excretion have continued. Drs. Davidson, Levinsky and Berliner have shown that during sodium diuresis the rate of excretion of potassium is unaffected by depression of the filtered potassium by up to 40%. These data are interpreted to indicate that the filtered potassium makes no appreciable contribution to the excreted potassium and that the filtered potassium is totally reabsorbed before the potassium destined for excretion is added by exchange for sodium.

Work of Drs. Orloff, Wagner and Davidson showing that increasing potassium excretion decreases the free water clearance indicates that the site of potassium secretion is in the same region of the tubule as that in which the reabsorption leads to formation of dilute urine. The data are in accord with the view that the increasing secretion of potassium substitutes cation exchange for combined reabsorption of cation and anion.

Drs. Orloff and Rector have continued their studies of factors influencing the rate of ammonia excretion. A reliable method for measuring blood ammonia has been developed after many attempts to apply methods described earlier. Application of this procedure to renal venous and arterial blood in dogs subjected to acidification and alkalinization of the urine has established that renal ammonia production changes much less than ammonia excretion, increased amounts being added to renal vein blood when urinary excretion falls. Attempts are currently being made to extend these studies, done on anesthetized and rapidly deteriorating animals, to unanesthetized animals previously prepared surgically. Investigations of renal enzymes possibly involved in ammonia synthesis are continuing in an attempt to identify those involved in adaptation to acidosis in the dog; the increase in glutaminase activity with acidosis in the rat has been found to be absent in the dog.

Drs. Burg and Orloff have investigated the effects of strophanthidine in the chicken. When infused in one leg so as to be delivered via the renal portal vein in relatively high concentration to the tubules

of the kidney on the infused side, doses of 0.5 mg/min. produce a marked impairment of sodium reabsorption with some inhibition of potassium secretion and interference with acidification. These studies support the view that the action of the cardiotonic glycosides is attributable to interference with electrolyte transport processes generally including those in the heart muscle fibre.

Studies of phosphate excretion and the effects of parathormone in the chicken have been completed by Drs. Levinsky and Davidson. These experiments have, for the first time, demonstrated a direct effect of parathyroid hormone on renal phosphate transport and the existence, at least in the chicken, of a secretory process for phosphate.

Dr. Kennedy has conducted studies of the diuretic activity of the new orally administered compound, chlorothiazide. These have shown the drug to be an active diuretic with which moderately severe cardiac patients can be maintained free of edema despite moderate intakes of salt. However, these investigations have not substantiated current reports that chlorothiazide is highly effective in highly resistant patients nor that it compares favorably with parenterally administered mercurials. Several patients who were totally unresponsive to chlorothiazide have responded well to mercurial diuretics.

Further insight into the genesis of elevated urine CO_2 tensions and the effect of buffer concentration has been acquired from kinetic studies and a theoretical computation by Drs. Kennedy, Eden(Laboratory of Technical Development) and Berliner. These indicate that the rate of CO_2 formation after addition of acid to bicarbonate containing solutions is kept low, in the presence of buffer, because of the minute concentration of carbonic acid which must drive the reaction to CO_2 and water. This presumably permits the escape of the urine from the tubules before attainment of reaction equilibrium.

2. Ion movements across biological membranes: Dr. Cotlove's studies of the distribution of ions in tissues have been held up pending the development of improved methods of analysis. This preliminary work has now been completed and has resulted in a new apparatus for the simple, rapid and almost foolproof determination of chloride. The instrument is now available commercially.

Drs. Hajdu and Leonard (Laboratory of Cardiovascular Physiology) are continuing attempts to identify the components of a system, present in plasma, which exerts an effect on the heart similar to that produced by the digitalis glycosides. This system, which originally excited interest because of its increased concentration in the plasma of hypertensives, has proved to be highly complex, consisting of at least two protein components and several smaller elements, one of them lipid-soluble and one calcium. Of these only a large protein component with a high affinity for heart muscle is present in increased amounts in hypertensives.

Dr. Dunham has investigated further the energy sources for cation transport in the human red blood cell. In glucose depleted cells and cells poisoned with iodo-acetic acid there is an excellent correlation between the utilization of high energy phosphate and active potassium influx. Attempts to reverse the strophanthidin inhibition of active transport with adrenal steroids (desoxycorticosterone and 2-methyl fluorohydrocortisone) have been unsuccessful.

Drs. Hogben and Cooperstein have initiated studies of salt and water transfer across intestinal membranes of the frog. In the large intestine there is a considerable electrical potential attributable to active transport of sodium. The movement of chloride is apparently passive and due to the potential and is abolished when the potential is short-circuited. Interestingly, there seems to be a considerable component of "exchange diffusion" involved in the chloride movement. Dr. Cooperstein with Dr. Brockman (Laboratory of Cardiovascular Physiology) has initiated parallel studies of loops of intestine in the unanesthetized dog. Dr. Hogben has extended his studies of anion transport across gastric mucosa and, in collaboration with members of the Laboratory of Chemical Pharmacology, has investigated the absorption of drugs from the human stomach and from the stomach and intestine in experimental animals. All of these observations can be interpreted as in accord with the hypothesis that drugs cross these barriers in the unionized, lipid soluble state and that the rate of movement is determined by their pK's and the pH of the solution from which the absorption occurs. The latter is not necessarily the pH of the bulk solution to which the mucosa is exposed, but is presumably a region in which a more fixed pH is maintained, e.g. the gastric tubules in the case of the stomach.

3) The disturbed physiology of congestive heart failure and experimental edema formation: These studies have been conducted by Dr. Davis with Drs. Ball and Yankopoulos and aimed at further elucidation of the disturbances which lead to the retention of sodium salts and water in animals with intrathoracic constriction of the inferior vena cava and with right sided heart failure due to constriction of the pulmonary artery. These have established the presence of increased amounts of aldosterone in adrenal venous blood as well as in the urine. Constriction of the inferior vena cava below the liver but above the adrenal veins has failed to produce edema or increased secretion of aldosterone, thus excluding adrenal venous congestion as an important factor. Attempts to locate a hypothalamic center modulating aldosterone secretion have not suggested, to date, the presence of such a center. Large lesions in various parts of the hypothalamus have failed to produce decreases in aldosterone excretion or increases in sodium excretion in a number of dogs with ascites due to inferior vena caval constriction. These studies are being continued with an examination of the acute effect of hypothalamic lesions on aldosterone output in adrenal venous blood.

Application of a body cast to animals with constricted inferior vena cava has, in several instances, resulted in diuresis and decreased aldosterone output. These studies confirm the importance of having an area for collection of fluid if elevation of venous pressure is to lead to sodium retention.

The development of a method for determining aldosterone on 10 ml of adrenal vein blood (Dr. Ralph Peterson, NIAMD) has permitted the design of acute experiments to study the acute effect of various procedures on aldosterone output. The increase in aldosterone secretion following constriction of the inferior vena cava begins within 15-30 minutes.

Drs. Yankopoulos and Davis have also recently started a study of the character of isolated heart muscle proteins and glycerol-extracted fibers from animals with experimental cardiac failure. The methods have been worked out and a few preliminary observations made. To date no difference has been found between material from normal dogs and those with experimental failure.

Laboratory of Cardiovascular Physiology

The energetics of the contracting myocardium. Activity in this long-term project for the past twelve months has consisted of a continuation of the studies outlined in the previous report, a comprehensive analysis of all available data and preparation for publication. The data obtained support the position outlined in previous reports and indicate that the oxygen requirement of the heart is not meaningfully related to the external work performed by it but that the oxygen requirement consistently correlates well with the Tension-Time Index (mean systolic pressure x the duration of systole). These data provide an objective basis for understanding the clinical observation that patients with heart disease in which stroke volumn and cardiac output are markedly elevated (mitral regurgitation, arteriovenous fistula, shunts) only rarely show overt evidence of myocardial hypoxia while patients with heart disease in which the myocardium is called upon to develop a large total tension (such as hypertension, aortic stenosis) do show such evidence in the form of angina pectoris.

An "amendment" to the basic Starling law concept (the energy of contraction is a function of end-diastolic fiber length) is being considered as a result of these data, namely that, while the end-diastolic fiber length determines the energy which can be put forth during the ensuing contraction, it is the nature of the contraction (tension or shortening) which determines how much energy is put forth. Current studies are directed to the acquisition of data with which to evaluate the validity of this "amendment."

The pathophysiology of experimentally induced mitral and aortic regurgitation. The techniques described in previous reports have been used in experiments which show that in mitral insufficiency a large mitral regurgitant volume (of the order of four liters per minute) can be sustained with only a minimal fall in forward cardiac output and aortic pressure, and associated with only a slight rise in mean left atrial pressure. The left ventricular function curve is only slightly depressed in the presence of large volumes of mitral regurgitation (2 to 3 times effective forward flow). The rise in left atrial pressure following an increase in systemic flow is substantially greater than the rise in left atrial pressure following a similar increase in mitral regurgitant flow.

In aortic insufficiency regurgitation is tolerated with considerable elevation of left ventricular end-diastolic filling pressure, but with relatively little rise in left atrial pressure. The ventricular function curve

is markedly lower after the production of aortic insufficiency than in the normal state; but if the regurgitant stroke work is added to the forward stroke work, the ventricular function curve is even higher after aortic insufficiency is produced. The differences between left ventricular end-diastolic and left atrial pressures could be diminished or almost abolished by inducing concomitant mitral insufficiency. However, the fall in left ventricular end-diastolic pressure was accompanied by a further diminution in effective stroke volume beyond that which had been produced by the aortic regurgitation alone.

The determinants of the duration and mean rate of ventricular ejection. The object of these experiments was to investigate systematically the effect of a variety of factors influencing the duration and mean rate of ventricular ejection in the isolated, supported, non-failing canine heart preparation. It was found that when stroke volume alone is increased, while all other parameters are held constant, the duration of ventricular ejection and mean rate of ejection are increased. An increase in heart rate at constant stroke volume decreases the duration of ejection per beat but increases the duration of ejection per minute as well as the mean rate of ejection. An increase in mean aortic pressure alone has little influence on either the duration or mean rate of ventricular ejection until markedly elevated mean aortic pressures are reached. Under such circumstances, duration of ejection is lengthened and the mean rate of ejection decreased. Hypothermia alone prolongs the duration of ejection, while sympathomimetic amines have the opposite effect. Unlike the non-failing heart, the failing heart exhibits an increase in the duration of ejection as stroke volume declines on the descending limb of its ventricular function curve.

The effects of atrial versus ventricular pacemaking on the heart's performance characteristics. It has been ascertained in preliminary experiments that ventricular pacemaking results in a substantial depression of the ventricular function curve when compared with atrial pacemaking or conversely at any given work level (compounded of specific flows, output pressures and rates) the ventricular filling pressure required is higher with ventricular than with atrial pacemaking and that under these circumstances the heart's oxygen utilization is higher with ventricular than with atrial pacemaking.

Development of a bioassay technique for local vaso-activity with satisfactory reproducibility. The lack of reproducibility of the flow

response to intra-arterially injected vaso-active agents had previously compromised its usefulness for this purpose. From the contour of some of the flow curves obtained it was suspected that lack of adequate mixing rather than changes in biological reactivity might account for the poor reproducibility. With this in view, the "intra-arterial" injections were made into a small multiple injection mixing cuvette (rotating magnet) placed in the femoral arterial flow line. Blue dye studies demonstrated the improved mixing thus obtained and the bioassay technique subsequently yielded gratifying reproducibility and adequate dose-response curve data.

The possible physiological significance of vasodilator substances in human urine. During the past year, an attempt has been made to isolate the substance responsible for the vasodilator activity present in urine and determine its physiological significance. It was found that there are at least two substances in normal urine (both dog and human) which can dilate blood vessels: an organic base which is dialyzable and appears to be histamine; the second material which is non-dialyzable and a protein has biochemical properties which seem to indicate a similarity of this substance to Kallikrein which was described by E. Werle in 1930.

A comparison of patients with orthostatic hypotension and normal controls reveals a decreased urinary excretion of the non-dialyzable component in the patient group. This was interpreted as suggesting either a) that the vasodilator substance in human urine is in some way associated with autonomic function since its presence is greatly diminished in patients with an established autonomic defect and/or b) that the vasodilator substance in urines mirrors the relative average blood level of such a substance which, in turn, is an expression of one facet of the physiological response to blood pressure regulation.

Studies on heart valve replacement. This project was reopened this year with a view to ascertaining whether the embolic phenomena which had precluded the clinical application of the apical-aortic anastomosis technique might be eliminated by the proper modification of the two points of attachment of the prosthesis at the ventricular apex and the aorta (see previous reports). It was found that impregnating a sleeve of stretchable dacron-orlon with elastic silicone and affixing this to the outflow end of the valve made it possible to perform an end-to-side anastomosis to thoracic aorta which appears to have overcome the difficulty. It has also been ascertained that the newly constructed silastic ball-valve produces substantially less hemolysis than the previously used ball-valve prosthesis.

The study of drugs affecting myocardial contractility by means of a new isolated atrium preparation. This project, begun in November 1956, was concerned with development of experimental techniques and apparatus until August 1957. Since then, a series of experiments has been run to explore the potentialities of the method developed.

The preparation consists of the left atrium of the cat or rabbit, made into a closed sac by tying the mitral ring around a cannula, ligating the pulmonary veins, and dissecting away all adjacent tissue. The atrium is filled with oxygen under pressure, and is mounted in a chamber filled with Krebs solution. It is stimulated at a constant frequency. In rapid succession, records are then made of the diastolic volume, stroke volume, and the pressure generated by "isometric" contractions, at a number of distending pressures. This permits the construction of pressure-volume curves, and "Starling curves" for both "isometric" and "isotonic" contraction from apparent ones produced by changes in frequency or diastolic fiber length.

It was observed that isolated left atria frequently developed a rapid spontaneous rhythm, and the necessity to suppress this in order to maintain a constant frequency of contraction prompted several experiments. It was found that the tendency toward auto-rhythmicity was increased by distention and by low potassium concentrations, decreased by increased potassium, pentobarbital, and reserpine. Reserpine pretreatment effectively prevents the production of auto-rhythmicity by the sympathomimetic amines without significantly affecting their inotropic effects.

The diastolic pressure volume characteristics of the preparation were not changed by any of the drugs tested so far except the cardiac glycosides in toxic concentrations. The "Starling curves" were not changed in shape, but were shifted up or down by drugs having positive or negative inotropic actions, respectively. The curves regularly exhibit a descending limb, and it is of special interest, from the theoretical point of view, that the peak of the curve, e.g. the diastolic volume associated with the strongest contractions, appears to be very nearly constant regardless of whether the curve is elevated or depressed.

Experiments on the isolation and characterization of a cardioactive protein system in serum. These studies conducted jointly with Dr. Hajdu of the Laboratory of Kidney and Electrolyte Metabolism are described with the work of that laboratory.

The effects of blood flow on transcapillary diffusion in mammalian skeletal muscle. The principal phase of the study of blood flow and vascular tone on transcapillary K^{42} exchange in the isolated gracilis muscle of the dog was completed. In general, the conclusion was reached that the exchange rate is determined principally by two factors: the total blood supply and the extent to which the capillaries are open to the flow of blood. Experimentally, it was observed that sympathetic vasoconstriction led to a greater fall in K^{42} influx than could be attributed to the fall in flow alone, and that active (and reactive) hyperemia led to a greater increase in K^{42} influx than could be accounted for by flow alone. Thus a direct effect on the number of open capillaries is suggested, in agreement with histological study. Attempts to study sympathetic vasodilatation were not successful.

Laboratory of Technical Development

The Laboratory's work in development of new and more sensitive methods and instruments is continuing with the evaluation of flame photometric methods versus electrodeless discharge methods for excitation of ultra-micro quantities of the alkali metals. The virtues of the flame photometer continue to be its simplicity and high sensitivity, but sample handling and dilution problems remain. The electrodeless discharge method remains the most sensitive.

Investigations are underway to extend the applicability of spectrophotofluorometry to the red and near infra-red, as well as to include phosphorescence emission spectra. An electrostatic method of storage and rapid read-out of these spectra appears to be sensitive enough to allow high efficiency flash sources to be used. These promise to provide higher outputs at the short wave-lengths and extend the utility of the method to the far ultraviolet, as well as into the red end of the spectrum. The read-out method allows easy correction of the emission spectra, without the use of cumbersome double beam methods.

The micro freezing point depression apparatus of Ramsay has been evaluated in relation to the possibility of reducing the size of the bath, and providing multiple samples, as well as freedom from thermometer calibration. A reproducible time-temperature cycle has been utilized to allow unknowns to be read as linear interpolations between

standards to eliminate the possible errors of absolute temperature readings. A trial apparatus has been constructed and the method shown to be practical, the current objection being the long time required for the temperature cycle to allow easy readability of multiple end points.

Several methods for improving the utility of gas chromatography methods by providing a more sensitive, more stable detector are being evaluated. Of the several methods tested, a new method utilizing a high frequency discharge at atmospheric pressure and a sonic velocity method have shown considerable promise in terms of sensitivity and stability. The drawbacks of the sonic instrument would appear to be its complexity, whereas for the radio frequency instrument the system remains moderately simple.

Micro pH electrodes in the 50 - 100 micron size range and the associated apparatus have been applied to physiological problems in collaboration with the Laboratory of Kidney and Electrolyte Metabolism.

Studies on the nature of the protein water-hydration structure by means of freezing and drying data have shown agreement with theoretical predictions in experiments on vacuum drying rates for gelatine and albumin. A micro fine (40 microns) thermocouple has also been developed for application to the problem of rapid freezing and drying research techniques, and promises to have other applications in biophysical investigation. A high speed self-balancing potentiometer utilizing the amplification inherent in the photomultiplier has been improved recently to provide more linear calibration and freedom from photo tube fatigue effects previously noted. An electron microscope has been acquired and essential techniques for the application of the instrument to freezing and drying studies have been completed.

The application of modern mathematical techniques to some of the problems of renal physiology has resulted in a better understanding of the mechanism involved in the problem of the carbon dioxide tension in plasma vs. urine and has contributed to the setting up of models for the explanation of physiological phenomena.

A mathematical analysis of the interchange of materials in multicompartment systems has revealed a hitherto unrecognized general relation which relates the transport through a capillary bed of a substance

which exchanges with the extra-vascular fluid compartment to the transport of one which does not. Utilizing this theoretical result – if the outflow curves are experimentally known it should be possible to obtain the law governing exchange between the capillary bed and the extra-vascular fluid compartment.

A special purpose analog computer for the analysis of overlapping distribution functions frequently encountered in biology and spectroscopy has been constructed. Preliminary experiments have shown that it is possible to analyze complex overlapping curves to permit higher resolution to be attained, as well as to provide an easy approach to the cataloging of these data. Two new professional members have been added to the Laboratory, Dr. A. Weissler, who will investigate biochemical effects of ultrasonic waves on the basis that it is expected to contribute to the understanding of biochemical reactions, as well as to provide a possible tool for controlling or studying these mechanisms. Progress to date has been largely setting up the apparatus and gathering basic preliminary data. It is hoped that Dr. Hayes' interest in enzymology will provide the Laboratory with the possibility of contributing to the development of methods for the study of enzyme reactions. Progress to date has largely been confined to setting up apparatus and methods.

The program for the development of prosthetic valves for the aortic and mitral area continues to indicate that a silicone rubber tricuspid valve may be a satisfactory prosthesis for insertion into the aorta. Technical difficulties in the production of these valves have prevented full evaluation of their performance. Several new molds just completed promise to provide a range of sizes for testing in animals. A very compact, all plastic, hydraulically operated pump provided with the new tricuspid plastic valves and two ventricular chambers has been constructed and subjected to preliminary tests for supporting the circulation of a dog during cardiac surgery. Features of this pump are atraumatic handling of the blood, low filling volume and the possibility that it might be made available in the form of a disposable presterilized heart pump package to facilitate either heart surgery or the support of an ailing organ for a temporary period of time.

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. A high quality CBS type color television chain including a cinescope recorder has been set up and provided with the necessary stand and accessories to allow it to be used conveniently with the microscope or special optical instruments for viewing the interior intact human eye. Guest researchers have been invited to test the equipment on their particular problems and evaluate the records obtained. Studies of micro circulation of frog kidney, rabbit lung and the retina of the human eye have been completed and the result recorded on film. The use of this apparatus provides high sensitivity, thus reducing the amount of light energy necessary to make the observations and providing the investigator with controls of contrast and color that otherwise are not obtainable. Methods of providing specific absorptions with assigned colors have been given a cursory test. At the present time it appears this equipment offers several important features not easily obtainable; the maximum utilization of the equipment requires special high sensitivity tubes and the continuous services of a competent technician to keep the apparatus in shape.

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 Clinical Biochemistry

Studies on serotonin. One of the important mechanisms for inactivating serotonin is the enzyme monoamine oxidase (MAO). This enzyme is present in most organ tissues and is found in highest concentrations in the mitochondria. It had not previously been possible to solubilize mitochondrial MAO oxidase. However, it has now been shown

that an appreciable amount of MAO activity is found in the soluble fraction of homogenates and that this activity resembles mitochondrial MAO in substrate specificity, pH optimum, etc. Soluble MAO has now been purified 20-30 fold and resolved into two components, the deaminase fraction (I) which converts serotonin to 5-hydroxyindoleacetaldehyde and the aldehyde dehydrogenase fraction (II) which in the presence of DPN oxidizes the aldehyde to 5-hydroxyindoleacetic acid. Using purified deaminase it has been possible to generate sufficient amounts of the aldehyde to study its chemical properties. It will be interesting to see if this aldehyde possesses physiologic activity of its own. The deaminase has been studied and attempts are being made to determine the nature of the coenzyme. It is hoped that such studies will yield information concerning the mechanism of action of the known MAO inhibitors and will permit the synthesis of even more potent inhibitors for in vitro and in vivo use. A large number of compounds have been screened for activity as MAO inhibitors and many known drugs were found to be quite active, including procaine amide and mephanesin. In addition it has been found that substitution of the indole ring in the 2-position almost invariably leads to active MAO inhibitors. Unlike the substituted hydrazides the inhibition produced by the 2-substituted indoles is readily reversible.

The most widely used MAO inhibitor, marsilid, was previously shown to be ineffective in blocking the metabolism of injected serotonin in rats and mice. Even more potent hydrazine inhibitors of MAO are incapable of blocking metabolism of serotonin, in vivo. It was first considered likely that marsilid and its analogues are not very active in vivo. It now seems more likely that there is another major route of amine metabolism which is not blocked by marsilid and its analogues and which is destroyed during homogenization.

Marsilid also appears to influence aldehyde metabolism, which may account for its ability to diminish the excretion of 5HIAA. It may do this by interfering with DPN enzymes one of which is aldehyde dehydrogenase. The actions of alcohol, which is also metabolized by a DPN enzyme, are potentiated by marsilid. These effects will be investigated. In addition the metabolism of marsilid will be studied to see whether any of its metabolites are responsible for this action. A method for marsilid assay has already been developed.

Further studies on release of serotonin during anaphylaxis indicate that although platelet serotonin is readily released other serotonin depots are not. It remains to be seen whether serotonin release plays a significant role in anaphylaxis.

Biosynthesis of indoleacetic acid. Indoleacetic acid (IAA) is present in normal human urine to the extent of about 5 mg/day. IAA excretion has been found to be markedly increased in Hartnup's syndrome and sprue. Increased levels in other patients have been observed with no apparent correlation to clinical findings. One patient with sprue was found to excrete 200 mg. of IAA per day. Since this was markedly reduced by antibiotics it was apparent that intestinal bacteria were responsible for the unusually large excretion. The culture of bacteria from his gut yielded organisms which readily formed IAA from tryptophan and ketoglutarate. However, tryptamine forming bacteria were also demonstrated.

Administration of large amounts of tryptophan to normal individuals produced a marked rise in IAA; only a small portion of this IAA production was affected by antibiotics indicating that it can be formed by mammalian tissues. In vitro studies with tryptophan showed that in the presence of O_2 it is converted to IAA. Since pyridoxal phosphate and α-ketoglutarate had little effect on the process it appears that the conversion is one of oxidative deamination to indolepyruvic acid followed by oxidative decarboxylation to IAA.

Studies on adrenaline, noradrenaline and related catechol compounds. Much remains to be learned concerning the formation and metabolism of the catecholamines.

Pheochromocytoma tumors have been shown to be extremely rich in the system which catalyzes the oxidation of 3, 4-dihydroxyphenylethylamine to noradrenaline. This tissue will be valuable in studies on the mechanism of the side chain oxidation.

As for metabolism, the soluble monoamine oxidase preparation (see serotonin report) has been applied to adrenaline and noradrenaline, making it possible to produce milligram quantities of the intermediate aldehyde (3, 4-dihydroxyphenylglyoxal) and the acid end product (3, 4-dihydroxymandelic acid). The chemical properties of these compounds have already been investigated. The possibility that the aldehyde possesses pharmacologic activity is to be investigated.

A pathway of catechol metabolism, heretofore unsuspected, involves methylation of the 3-hydroxy group. The acid end product of this route of metabolism (3-methoxy-4-hydroxy-phenylglycollic acid) was first demonstrated in urine by Armstrong. Subsequent work in this laboratory and by Axelrod in NIMH has shown that almost all catechol compounds including noradrenaline and adrenaline can undergo this O-methylation. The 3-methoxy analogue of noradrenaline has been found in human pheochromocytoma, its first demonstration in man. The physiologic significance of O-methyl analogues of the catecholamines remains to be determined. It may well be that O-methylation of noradrenaline is the limiting step in its action and that oxidative deamination follows secondarily.

Metabolism of γ-aminobutyric acid. γ-Aminobutyric acid (GABA) is formed by enzymatic decarboxylation of glutamic acid. In animals the highest concentration of GABA and of glutamic decarboxylase occur in the central nervous system. Recently GABA has been claimed to be a regulatory substance in the brain and drugs structurally related to it have been known to have marked sedative action.

In studying the metabolism of this compound in vitro it has been found that arginine can transfer an amidine group to GABA converting it to γ-guanidinobutyric acid. The enzyme for carrying out this transamidination appears to be the same as that required to amidinate glycine to form guanidinoacetic acid. Of a large number of amino acids investigated only glycine and GABA are amidinated to an appreciable extent. Dr. Irreverre, NIAMD, has shown that γ-guanidinobutyric acid is found in brain and other tissues and is in fact the only guanidino acid present in detectable amounts in tissues.

A sample of the guanidino acid was sent to Dr. Harry Grundfest at Columbia University who reported that it is a potent convulsant agent, in his test system, indicating that guanidination converts GABA from a sedative to a convulsant type compound. The significance of this route of GABA metabolism will be further explored as will other metabolic routes.

Biogenesis and metabolism of aromatic compounds. Although it is known that enzymatic hydroxylation of aromatic compounds to phenols, i.e., phenylalanine, aniline and kynurenine, requires reduced

pyridine nucleotide Fe^{++} and oxygen, little else is known concerning the mechanism. One of the conceivable mechanisms would involve direct addition of the elements of H_2O_2, to yield a dihydrodiol compound. Actually such compounds are formed by the microsomal hydroxylating system from naphthalene and apparently from quinoline. Recent studies indicate that these dihydrodiols, though they are enzymatically formed and chemically yield the appropriate phenols, do not appear to be intermediates in phenol formation. The significance of dihydrodiol formation is being investigated. Further studies with tritium water may yield additional information concerning mechanisms involved in phenol formation.

Apparently phenylalanine or one of its metabolites can be hydroxylated to form an ortho substituted phenol, the final product being o-hydroxyphenylacetic acid (OHPAA). Two suspected intermediates, ortho-tyrosine and ortho-tyramine, have thus far not been detected in animal tissues. The central activity of o-tyrosine and the large excretion of OHPAA in phenylketonuria make this pathway an interesting one. Attempts are being made to determine whether there exists a specific o-tyrosine decarboxylase and to detect o-hydroxylation of phenylalanine in in vitro systems.

Conversion of non aromatic to aromatic compounds takes place readily in animal tissues and there are products in foods which undergo aromatization, i.e., quinic acid. The conversion of hexahydrobenzoic acid to hippuric acid was shown to be catalyzed by an enzyme system in liver mitochondria requiring CoA. By synthesizing hexahydrobenzoyl CoA it was possible to show that a condensation with glycine was required to drive the aromatization reaction.

Studies on hydroxyproline. Hydroxyproline (OPr) is an unusual amino acid in that in animals it is incorporated solely into connective tissue proteins, i.e., collagen and elastin. Because of this unique distribution its metabolism should reflect changes in connective tissue protein metabolism.

Using OPr-C^{14} it has been shown that this amino acid can be readily incorporated into rapidly developing connective tissues, such as developing chick embryos and artifically induced guinea pig fibrous tissue. This finding is contrary to the currently accepted belief that hydroxylation of proline occurs after incorporation of proline into a

pro-collagen moiety. Additional support for the direct utilization of hydroxyproline is the demonstration of conversion of proline to free hydroxyproline by tissue homogenates.

Studies on the influence of a variety of factors on the OPr content of tissues, blood and urine have also been of interest. Thus it was shown that ascorbic acid while preventing connective tissue formation in guinea pigs, did not influence the levels of OPr in blood and urine Studies on patients have shown that little exogenously administered OPr is normally excreted in the urine even when several grams of the amino acid are administered. However, certain patients (Marfan's and Erlos Danlos syndrome) tend to spill appreciable quantities of a loading dose into the urine. Elevated levels of OPr excretion were also observed in a few other clinical conditions. Of interest, too, is the finding that administration of glutamic acid (no other amino acid) along with OPr causes the latter to be excreted in the urine.

Analogues of OPr, ketoproline and thiomethyl proline, raised free OPr levels in the developing chick whereas acetyl salicylate and cortisone lowered them. Ketoproline also blocked the destruction of OPr in homogenates. These findings may be of therapeutic interest.

General Medicine and Experimental Therapeutics Branch -- Experimental Therapeutics Section

The Section of Experimental Therapeutics has been interested during the past year in many projects alone and in collaboration with other groups in NHI and NIH. The principal projects may be grouped as (1) Studies on Vasoactive Substances, (2) Metabolism of Amino Acids in Man, (3) Action and Metabolism of Drugs and (4) Studies on Anaphylaxis.

(1) Vasoactive Substances -- Studies on patients with the carcinoid syndrome have shown clearly that there is no difference in plasma serotonin in mixed venous blood and arterial blood to explain the predominately right heart lesions. The administration of iproniazid (marsilid) to one patient produced a marked aggravation of symptomatology.

C^{14} labeled norepinephrine was administered to a patient with pheochromocytoma and showed a turnover rate of a few hours in contrast to days required for turnover in the adrenal gland. The recently described methoxy metabolites of epinephrine and norepinephrine are being studied as are other possible metabolites of these amines. O-methylated metabolites have been shown in dogs to be relatively inactive. This suggests that this metabolic pathway is a means of physiologic inactivation. Studies are being pursued in an effort to improve the present chemical techniques of determining blood and urine epinephrine and norepinephrine levels. The differential pH oxidation procedure is being studied and is giving promising results.

Various mast cell tumors in animals and in patients with urticaria pigmentosa have been studied in relation to histamine and serotonin release. Extensive biochemical studies are being carried out on these tumors and the effect of various drugs on the release of histamine and serotonin are being observed.

(2) Metabolism of Amino Acids -- Because of its relationship to connective tissue (especially the collagen protein) interest has been stimulated in the metabolism of hydroxyproline. During the past years studies have been made in 71 individuals, 9 of whom were patients with Marfan's Syndrome. In 7 of these 9 patients, the urinary excretion of hydroxyproline has been abnormally high. Excretory studies have been performed when hydroxyproline is given alone or in combination with gelatin or casein.

Indoleacetic acid has been studied in urine and tissues. Normal daily levels of excretion were found to be 4.0 to 18.0 mgm/day; 5 patients with apparently unrelated disorders (diabetes, idiopathic sprue, cerebellar ataxia and amytrophic lateral sclerosis) showed elevated rates of excretion. Sterilization of the gut with antibiotics caused considerable alteration in patients with both normal and abnormal rates. These observations will be extended to establish the significance of these abnormal values and in an effort to understand more completely the metabolism of tryptophan.

(3) Action and Metabolism of Drugs -- This program has been fairly quiet during the year. Preoccupation of the investigators with other more pressing studies, the departure of two members of the professional staff, and the lack of outstanding compounds for clinical trial

have resulted in only minor studies in this program. Some significant observations have been made on iproniazid (marsilid) and mecamylamine. It is expected that more work will be done in this field during the coming year.

(4) Studies on Anaphylaxis -- As reported in the past, it has been shown that histamine and serotonin are released during anaphylaxis in rabbits. More recent studies have shown that the lung level of serotonin is markedly elevated immediately after the injection of the antigen. Studies in other animals and in man have shown large amounts of histamine and relatively little serotonin in the lungs. It has been suggested that serotonin might be an important factor in pulmonary embolism. Preliminary studies in dogs have failed to show any significant serotonin release in experimental pulmonary embolism.

Recently, studies in hypersensitivity in humans have been instituted in collaboration with Dr. Halla Brown of George Washington School of Medicine. Since serotonin is released in animals during anaphylaxis it is suggested that this factor may be important in hypersensitive states in man. Reserpine therapy has been administered in 80 patients with hay fever in an effort to deplete the serotonin stores and lessen the hypersensitive reaction. These studies are to be pursued and other studies related to histamine and its metabolites are planned or in progress.

(5) Other studies -- Clinical studies on plasma proteins have been performed in collaboration with Dr. Gordon, Laboratory of Cellular Physiology and Metabolism, and Dr. Jesse Steinfeld, NCI. The abnormality of protein metabolism in regional ileitis and ulcerative colitis has been related to protein loss into the lumen of the G.I. tract. These studies will be extended to clarify the picture and with the hope that isotopic PVP may be developed into a generally applicable diagnostic test for enteritis or colitis.

A few studies have been performed in collaboration with Dr. Joseph Bell, NIAID, in relation to the cardiovascular complications of influenza. In a study of infected prison volunteers no significant C-V abnormalities were found. These studies will be pursued as appropriate clinical material becomes available.

General Medicine and Experimental Therapeutics Branch -- Cardiodynamics Section

The Section on Cardiodynamics has continued its studies along three principal lines: (1) Studies in the field of electrocardiography, with particular emphasis on vector methods of interpretation, (2) hemodynamic studies with particular respect to studies of valve gradients as determined at cardiac catheterization or surgery, dye dilution techniques for diagnostic purposes, and other physiologic studies of the circulation, and (3) studies in the field of pulmonary physiology with particular emphasis on cardio-pulmonary problems. The principal studies are:

Electrocardiography -- A study is nearing completion of the Wolff-Parkinson-White syndrome, comprising seventy cases. It was found that a ventricular conduction defect is frequently associated with WPW and is due to invasion of left bundle subdivisions by the pre-excitation wave. Interestingly enough, the P-R interval duration governs whether or not the conduction defect will be present, and in one subject we could produce the ventricular conduction defect at will by manipulating the P-R interval.

The revised edition of Dr. Grant's book on vector electrocardiography was completed to be published this fall. Grollman's Textbook, "Clinical Physiology," in which the chapter on ECG was prepared by this laboratory, was published in January. A manuscript "Left Axis Deviation" was submitted to Modern Concepts of Heart Disease at their request.

New work initiated includes a systematic study of the electrocardiogram in congenital heart disease, using intracardiac leads.

During the month of October, while Dr. Grant was a visiting scientist at the University of Washington Medical School, experiments were done with Dr. Alan Scher of the Department of Physiology where for the first time lesions of selected parts of the left bundle system were proven capable of producing axis deviations and ventricular conduction defects without prolonging the QRS interval. This is an important finding in the small world of electrocardiography because it confirms an hypothesis introduced by this section that left and right axis deviations are due to ventricular conduction defects and not to variations in the anatomic position of the heart.

Hemodynamics -- In association with the Clinic of Surgery dye dilution methods have been developed for (1) measuring cardiac outputs simultaneously with pressure measurements to determine the adequacy of commissurotomy at the operating table, (2) qualitatively measuring aortic insufficiency, (3) detecting intracardiac shunts. Three reports on specific aspects of these studies have been completed for publication. A symposium, presented jointly by this section and the Clinic of Surgery, has been accepted for publication in Circulation entitled "Diagnostic Methods in the Study of Left-to-Right Shunts." A study of four cases of "right-sided heart" done jointly by the Clinic of Surgery, the Pathology Department and this section is nearing completion and defines for the first time in the English literature the dextroversion syndrome as a cause of right-lying heart, and as altogether different in etiology and manifestations from mirror-image dextrocardia.

The section "Heart Disease (Medical)" was written for 1958 edition of Annual Review of Medicine by Dr. Grant and Dr. Fox.

Cardio-pulmonary Studies -- A group of patients with advanced chronic pulmonary emphysema have been studied with relation to the stress distribution in the tracheo-bronchial tree. It is hoped that these studies will help in the understanding of the basic pathologic physiology of this disease. In addition, animal studies are being performed on dogs and on excised monkey lungs in an effort to define certain pressure relationships in the flow of gases in the tracheo-bronchial tree. In man, the compliance of the lung is being studied in relation to pulmonary vascular pressure in an effort to determine the effect of these pressures on pulmonary function. Normal subjects, in addition to patients with cardiac or pulmonary disease, are under study.

Studies are continuing with the catheter tip method of measuring blood velocity. An electronic analogue computer has been devised which will carry out instantaneous computations and the system gives fair results in animals. More critical studies in animals are planned before the method is applied in man.

A new type respiratory flow meter has been devised which has a low dead space and is immune to condensation effects. Many of the

technical aspects of this problem have been solved, thus making a significant contribution to this field of study. This work has produced a method for measuring maximum air velocities for all points of the vital capacity. From mathematical treatment of the curve representing these velocities it has been possible to make quantitative approximations of certain physical properties of the lung responsible for ventilatory abnormalities. For example, the location and dimensions of the obstructive component responsible for emphysema in a unit alveolus have been defined by this method.

Pharmacodynamic studies of the pulmonary circulation are in progress utilizing various secondary amines supplied by pharmaceutical firms. Suggestive evidence of pulmonary vasodilatation was noted with methoxamine and this substance will be studied further. Several other amines were shown to actually produce pulmonary vasoconstriction. These studies are also providing certain information which may be helpful in our understanding of the basic pathologic physiology of pulmonary hypertension.

General Medicine and Experimental Therapeutics Branch -- Clinical Endocrinology Section

The work of this section during the past year has been directed toward three groups of problems: a) the role of steroids in sodium retention, b) the function of parathyroid hormone and c) a group of studies not directly related to the above.

a) The role of steroids in sodium retention -- These studies have involved 1) investigation of improved techniques of aldosterone determination, 2) continued investigation of the normal mechanisms of control of aldosterone secretion, 3) continued investigation of the stimuli effective in controlling aldosterone secretion in pathologic states characterized by sodium retention and 4) investigation of the mode of action on the kidneys of aldosterone and other steroids.

1) A salicylyl hydrazide reaction was adapted to the determination of aldosterone in biologic fluids, and its application to physiologic studies begun. Simultaneously, the method of Neber and Wettstein for the determination of aldosterone was set up, and improved by addition

of a radioactive tracer to measure recoveries, and studies were begun on the use of the double labelling technique of Peterson. The presence of aldosterone in efferent blood from calf adrenals perfused with progesterone-C^{14} was proved by the Peterson method.

2) Studies on the normal mechanisms of control of aldosterone secretion were centered chiefly on the experimental alteration of intravascular volume and of posture. The blood volume was contracted by phlebotomy and expanded with red cells or salt-poor albumin: These stimuli were consistently effective in raising and lowering, respectively, the aldosterone secretion.

The supine posture was observed to lower the rate at which aldosterone secretion rose following salt deprivation, as compared to the rate observed in the erect posture.

3) In cirrhosis with ascites, expansion of the intravascular volume was found to lower aldosterone secretion markedly, but not to "normal" levels; potassium deprivation lowered aldosterone secretion without producing sodium diuresis. In nephrosis, the diuresis associated with meticorten therapy was associated with marked lowering of aldosterone secretion. In dogs, constriction of the inferior vena cava was inconsistently effective in raising the aldosterone content of adrenal vein blood; the effect of experimental alteration of blood volume on this response is under investigation.

4) The effect of aldosterone and of related steroids on the renal excretion of water, hydrogen, potassium, and sodium was studied both in human subjects with hyperaldosteronism and with Addison's disease and in adrenalectomized dogs.

b) The function of parathyroid hormone -- These studies have involved 1) attempts to develop an assay method for parathyroid hormone using the chicken and 2) attempts to evaluate parathyroid function in subjects with hyperparathyroidism.

1) In the studies with the chicken it has not thus far been possible to show a reproducible dose-response relationship in PO4 excretion when the kidney with parathyroid extract infused into the renal portal circulation is compared with the control; studies are in progress attempting to show a consistent relationship of urine P to hormone dosage.

2) Parathyroid function was assessed in normal subjects and in subjects suspected of hyperparathyroidism in four ways: a) The effect of intravenous calcium infusion on urine phosphate excretion. In all normal subjects, urine PO4 fell, in all subjects now known to have had primary hyperparathyroidism, it did not. b) The T_M of P. In all normal subjects the T_M P was above 3 mg per minute; in three patients with hyperparathyroidism it was below. However, seven patients with hyperparathyroidism had T_M P in the normal range. c) The effect of P deprivation. In subjects with hyperparathyroidism P deprivation produced a fall of serum P, and a rise in serum and urine Ca; in normal subjects, it had none of these effects. d) The percent of P reabsorbed by the renal tubules was found to vary with the P intake, and not to serve as an index of hyperparathyroidism.

c) Studies unrelated to the above -- 1) The movement of larger molecules through blood vessels and tissues is being evaluated by quantitative measurements of the rate of movement of I^{131} labelled albumin; capillary permeability coefficients for the various tissues studied have been determined. The effect of dicoumarol and other agents, and the corresponding data for other large molecules are being investigated.

2) The relationship of steroid structure of certain C19/esters with anabolic activity has been studied in man. It was found that removal of the nineteenth carbon from all steroids studied, either potentiated anabolic action or conferred anabolic activity where the "parent" steroid had none. The addition of 9α halogen (with 11β hydroxy) greatly increased anabolic potency of methyl testosterone.

3) A method for determining plasma testosterone was developed with the aid of radioactive tracers, and applied to the spermatic vein blood of the dog.

4) A new colorimetric method for serum and urine magnesium determination was developed.

5) The mechanism of the poor response of cirrhotic subjects to water loading was studied. Early results tend to invalidate earlier reports of increased antidiuretic hormone activity in these subjects.

6) The effect of estrogens upon the metabolism of hydrocortisone was studied by measuring both urinary and serum free and bound hydrocortisone before and after the administration of estrogens. The estrogens appear to interfere with the degradation of hydrocortisone to tetra-hydrocortisone, with resultant higher serum and lower urine levels.

Surgery Branch

The clinical investigative projects of the Surgery Branch during the past year have again utilized generally those patients with valvular heart disease and various forms of congenital heart disease with intra- and extracardiac shunts.

Several techniques have been used in the evaluation of patients with or suspected of having left-to-right shunts. The nitrous oxide test, previously described, has now been carried out in more than 400 patients. The usefulness of the test, as originally designed, has been proved and at the present time modifications concerning the concentration of nitrous oxide inhaled and the ideal sampling periods are being investigated. An outgrowth of the nitrous oxide test has been in the application of a radioactive gas for the detection of shunts. Two gases have been evaluated, trifluoroiodomethane and radioactive krypton. The latter gas, a beta emitter, seems most practical. At the present time parallel studies are being carried out involving both nitrous oxide and krypton. The advantage of the radioactive gas is that it can be analyzed almost instantaneously and the results of the catheterization can be reported while the procedure is still in progress. Yet another method for the study of left-to-right shunts has been the use of indicator-dilution curves from the left heart and aorta. When an indicator is injected into one of the chambers of the left heart or the aorta and a dilution curve recorded from a peripheral artery, the curve is normally characterized by an extremely fast ascent and descent. When there is a shunt or valvular insufficiency, there is a break or change in slope of the descending limb of the curve caused by regurgitation of a portion of the dye or its passage through the pulmonary circulation. Such studies have now been carried out in more than 100 patients with or suspected of having left-to-right shunts. In virtually all instances the technique has proved diagnostically accurate but with small shunts or

minimal degrees of insufficiency interpretation of the curve by gross inspection is difficult. Accordingly, methods for the quantification of the slope of the descending limb of the curve are now under investigation. A number of dogs were operated upon by Dr. Baker and various forms of left-to-right shunts constructed. When dye curves were performed in these animals with known types of left-to-right shunts the curves were in every instance diagnostic.

Left heart catheterization has continued to be an important and interesting part of the clinical work in the Surgery Branch. More than 700 transbronchial left heart catheterizations have now been carried out without significant sequelae. Previous reports have shown the usefulness of this technique in precise assessment of the degree of valvular stenosis and estimating the degree of valvular insufficiency. It has been shown that a catheter can be left in the heart while the needle and bronchoscope are removed. This has permitted us to make observations over prolonged periods of time and to make observations as to the patient's physiologic state. Determinations of oxygen consumption and cardiac output have shown that a steady basal state can be achieved within 20 minutes after the bronchoscope is withdrawn. Thus, observations can be made with the patient either in a basal state or during exercise. The use of norepinephrine infusion at the time of left heart catheterization has made possible the detection of minimal degrees of mitral insufficiency which could not be demonstrated in the left heart pressure tracings with the patient's blood pressure normal. As systemic resistance is increased by the infusion of norepinephrine, atrial pressure is elevated in the presence of mitral incompetence but not in the presence of a normal mitral valve or mitral stenosis. At the present time determinations of cardiac output are combined with norepinephrine infusion to determine the usefulness of this combined technique in the estimation of valvular regurgitation. Similarly, at the time of operations for valvular disease, it has been possible to measure the pressures simultaneously across the valve and to determine cardiac output. This is done before and after operations for the relief of valvular stenosis. Thus, the operating team is able to characterize precisely the effectiveness of the operation while still in the operating room and, if necessary, to perform a more adequate operation.

In the experimental laboratory, a special catheter has been designed which can be passed from the saphenous vein into the right atrium. The catheter contains a retractable needle which can then be extruded

and passed through the interatrial septum into the left atrium. Dr. Ross has catheterized a large number of dogs with this instrument and it seems a practical way of catheterizing the left atrium without the disadvantages of bronchoscopy or percutaneous cardiac puncture. The method has not yet been applied in patients.

Much laboratory and clinical time has been spent in the problem of extracorporeal circulation. One year ago a Melrose heart and lung machine was obtained and has been extensively evaluated in animals and in approximately 20 patients. Modifications of the instrument have been made to increase its efficiency and safety and, in the experimental animals, more than 80% survived 30 minutes of perfusion. With the application in patients, however, two serious problems have become apparent. These are massive postoperative bleeding, the cause of which is not at the moment clear, and the questionable capacity of the machine to provide flows adequate for patients weighing more than 40 or 50 Kg. Continued work with the Melrose oxygenator is being carried on in the experimental laboratory but more importantly, attention is being given to the development of a membrane oxygenator in which blood and gas are never allowed to come into direct contact. Aside from the development of an efficient membrane the problem of packaging this material in an efficient manner will have to be worked out. Physiologic studies are being carried out in patients operated upon with both hypothermia and extracorporeal circulation. This is a study concerning the cardiovascular hemodynamics following the closure of atrial and ventricular septal defects. Catheters are placed into the left and right sides of the heart before the defect is closed and are left in place after the chest is closed so that the pressures can be monitored during the postoperative period. Valuable information concerning the incidence of left heart failure following the closure of atrial septal defects and of right heart failure associated with ventricular septal defects has been obtained. It is planned to continue these studies.

Another clinical study involving both operating room records and those obtained at left heart catheterization has been the assessment of pulsus alternans in the left ventricle in patients with aortic valve disease. Approximately 20 patients have presented this phenomenon and studies to date indicate that this is a function of the tension developed by the left ventricle in overcoming the obstruction at the aortic valve. The importance of this phenomenon in selecting patients for operation is obvious.

Clinicians have speculated for many years about the actual volume of the regurgitant blood flow in aortic insufficiency. Experiments in the Laboratory of Cardiovascular Physiology have indicated that these may be extremely large. A method has been devised for estimating regurgitant volume in patients with aortic insufficiency. This is accomplished by passing a catheter into the aorta and injecting indicator dye at various levels while the presence of dye in the blood stream is monitored by a densitometer at the right ear. As the catheter is gradually withdrawn in the descending aorta the end point is determined from which dye regurgitates into the ascending aorta and appears at the right ear. From anatomic studies it has been possible to calculate the volumes of the aorta at various levels and thus arrive at a quantitative estimate of the volume of regurgitant flow. The procedure has been useful in assessing the degree of aortic insufficiency accompanying mitral and aortic stenosis and in differentiating aortic insufficiency from pulmonary insufficiency. The method has been established as a useful clinical tool and is being further refined in order to arrive at more precise quantitative estimates of regurgitant flow.

In the experimental laboratory several studies are underway concerning the sympathetic nervous system and its relationship to cardiac irritability and hypothermia. It has been shown previously that the injection of procaine in the region of the sino-auricular node is effective in preventing ventricular fibrillation in the hypothermic dog. The mechanism of this action has been obscure. Dr. Cooper has performed threshold stimulation tests on the ventricle with and without the procaine infiltration and has demonstrated that there is no change in the threshold to electrical stimulation. Similarly, the heart has been sympathectomized and the reactivity of the cardiac muscle to adrenalin and other adrenergic agents has been tested. Again no distinct difference could be detected in threshold levels before and after sympathectomy. An interesting outshoot from this work has been the demonstration that in hypothermic dogs, digitalis reverses the action of sympathomimetic drugs. The mechanism again is not clear but it has been possible to test this in several patients undergoing operations with hypothermia and it has been found to be true in patients as well as experimental animals. This particularly interesting aspect of the problem is being followed up.

With the use of the artificial heart and lung machine in patients, attention has been directed to the intracardiac and pulmonary hemodynamic changes associated with perfusion and particularly with elective cardiac arrest. During the period of cardiac arrest the bronchial arterial system supplies the pulmonary venous bed with relatively large amounts of blood. The influence of this blood flow on pulmonary venous pressure and on the distention of the heart has been the subject of investigation by Drs. Ross, Gilbert and others. It has been shown that with cardiac arrest it is absolutely essential that some chamber of the heart be opened in order to decompress the pulmonary venous bed. It has been shown that opening the right heart can adequately decompress the left heart through the pulmonary circulation. The study has obvious clinical application in the closure of intracardiac defects and particularly in the repair of aortic stenosis when none of the chambers of the heart would ordinarily be open.

Gerontology Branch

The work of the Gerontology Branch is concerned with identifying and describing changes in physiological and psychological capacities of aging humans. In addition, programs are being initiated to investigate the basic biological and biochemical processes of aging. These studies utilize cells and tissues from a variety of animal species.

In the area of human physiology, studies on the thyroid have been extended. Although the total capacity of the thyroid gland to accumulate iodine is not influenced by age, the rate of uptake during the initial stages of exposure to I^{131} is slightly reduced at advanced ages. There is also a progressive slowing in the rate of disappearance of intravenously administered thyroxine from the blood, although the level of protein-bound iodine in the plasma is not influenced by age. It has been estimated that in 80-year old subjects, 39 micrograms of thyroxine are degraded per day as compared with 62 micrograms per day in 50-year olds. It is concluded that the uptake of I^{131} by the thyroid gland and the protein-bound iodine content of the plasma are inadequate indices to detect variations in the normal range of thyroid function.

Mathematical analysis of simultaneously recorded pressures, at a series of paired intra-aortic and arterial locations, indicates that, in

the propagation of pressure in the human, factors of damping and reflection must be considered. Reflection is significant for low frequency components which represent a large share of the pressure energy. This fact precludes the accurate interpretation of pulse wave velocity measurements. Damping is significant for high frequency components.

Estimates of cardiac output during standardized arm exercise show a gradual decrement with increasing age that is in accord with the previously observed age reductions in resting cardiac output. The increased oxygen uptake observed during exercise usually exceeds the amount contained in the additional blood pumped by the heart. Hence, it is probable that older subjects react to physical exercise by diverting more blood from other organs into the exercising area. It has been possible to develop a technique for simultaneous estimation of cardiac output and renal blood flow so that some direct evidence can be obtained on this question.

The increased ventilatory response observed in older subjects following exercise is due, in part, to a reduced elimination of CO_2 relative to the ventilation volume. Increased pulmonary dead space in the aged contributes only slightly to the increased respiratory volume during exercise. Further studies of the maximum diffusing capacity of the lungs are being planned. In aged subjects, the extra work required for the augmented ventilation accounts for a large share of the extra oxygen taken up during recovery.

A more detailed analysis of arm motion shows that less energy is utilized in simultaneous contraction of opposing muscle groups when the amplitude of the motion is large than when it is small, even when both are performed with maximum torque. These studies have important implications in understanding the mechanism of the reduced capacity for work commonly observed in the elderly.

For the most part, descriptions of age changes are based on the average of values obtained on different subjects at each age level. For a more complete understanding of aging, it is essential that measurements be made on the same subject as he ages. Such longitudinal studies have been started and observations at 3 to 5-year intervals are being made with respect to renal function, resting cardiac output, pulmonary function and basal metabolism. Thus far, no striking changes have been found in the relatively few subjects tested over a 5-year interval.

A micro-osmometer has been designed and built which will permit a study of age differences in the response to an oncotic load.

Previous studies have indicated that age changes in physiological functions may be due, in part, to gradual losses in functioning tissues, as well as to changes in cellular function and metabolism. It is, therefore, important to develop indices of the amount of tissue present in the intact animal. Preliminary estimates, based on measurements of fat thickness, on chest x-rays show an agewise increase. Calculations indicate that fat free weight decreases more rapidly with age than does total body weight. A more detailed study is in progress which will utilize the helium displacement method for determining body volume in the intact human. The method has been set up and calibrated. It has an accuracy of 2%. Estimates of body volume permit calculation of the specific gravity which is related to the amount of fat present in the body. These estimates of body fat will be compared with calculations based on total body water determinations made with antipyrine.

In collaboration with the National Institute of Mental Health, a psychology unit has been established in the Gerontology Branch. During the year, two additional professional psychologists have been recruited and research programs have been formulated to study age changes in memory, verbal functions and the physiological aspects of attention and motivation. Arrangements have been completed to recruit subjects living in the community for experimental subjects. A number of exploratory studies, for the standardization of techniques, to determine age differences in the interpretation of words have been carried out. Apparatus for the recording of physiological responses has been assembled and standardized for use in experimental situations.

Studies on the Biology of Aging. -- Additional experiments have confirmed the agewise decrease in the succinoxidase activity of whole homogenates of kidney tissue in excess of the reduction in protein nitrogen. The decrement in succinoxidase activity is manifested by a decrease in both the rate of oxidation and of phosphorus esterified. However, the efficiency of this metabolic process, as measured by the P/O ratio, is unaffected by age. Since no agewise change in succinoxidase activity of isolated mitochondria could be demonstrated, it is probable that the reduced activity observed in the homogenates from the kidneys of old rats can be ascribed to a loss of mitochondria from the tissue. Similar experiments were performed on liver tissue, but no age differences were found.

The capacity of the liver to synthesize specific proteins was tested in old and young rats. Estimates of tryptophane peroxidase activity in liver of rats, stimulated by the intraperitoneal administration of tryptophane, showed a 6-fold increase in both old and young animals. Thus, the liver of the old rat showed no significant change in ability to perform a rapid, short term protein synthesis.

Although the mass of skeletal muscle in the rat diminishes with age, at a rate greater than the total body weight, there was no evidence of a similar reduction in brain weight or cardiac weight.

A microspectrophotometer has been assembled which permits the recording of spectral absorption characteristics of identifiable parts of individual cells. This technique is being applied to define the physical properties and ultimately the nature of substances which accumulate in the cells of old tissues. In addition, the fluorescence properties of these substances are being examined.

The use of fluorescent dyes to localize various compounds in individual cells is being explored.

Studies on the effect of temperature on aging have been initiated, utilizing Drosophila as the experimental material. Colonies of flies are being maintained at different temperatures and complete mortality curves are being worked out. These experiments have an important bearing on the theory that aging is related to the "rate of living." Although increases in environmental temperature have been shown to reduce life span in flies, it is not known whether this is due to a general augmentation of metabolic rates or to chance deviations in metabolism. Luminous bacteria offer an opportunity to investigate further the effects of temperature changes on viability.

Differences in the specificity of proteins in old and young tissues are also being investigated by immunological techniques. Work is in progress to determine the number, variability and distribution of antigens as a function of age and other variables. For these experiments, chickens have been injected with soluble muscle proteins from old and young rats.

A theoretical analysis of age specific mortality rates has been carried out. It has been shown that the logarithmic increase in

mortality with age exhibited by many species of animals can be accounted for by assuming that vitality (the ability to survive) decreases linearly with time, and that environmental challenges, which threaten existence, are distributed in magnitude according to the Maxwell-Boltzmann law.

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PHS - NIH
NATIONAL HEART INSTITUTE
Individual Project Report
Calender Year 1957

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; the Center for Aging 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 1957 which, it is hoped, have improved the overall functioning of the Heart Institute:

1. During the summer of 1957, a professor of public administration from a local university was employed as a consultant to the Heart Institute. His assignment while here was to make a detailed study of the Grants and Training Branch. As a result of this study and his recommendations, changes were made in the organization and procedures of that branch that have resulted in increased operating efficiency and program control.

2. A seminar was conducted by the same consultant, for members of the NHI administrative staff plus a small professional group. It was the feeling of those who participated that they had benefited greatly from new approaches to their work suggested in these discussions.

3. A Heart Institute personnel office was set up when the central NIH Personnel Office assigned a generalist to be located physically in the Institute. This generalist and two NHI staff members assigned to assist him are working closely with personnel of the Institute to administer a more effective and well-rounded personnel program.

4. A member of the administrative staff (Research) has participated in a management intern program of the Civil Service Commission for the past six months studying "Automation." It is expected that his training will be immensely helpful in enabling the Heart Institute to use to best advantage the facilities that will be provided by the new NIH computer.

5. The 1957 summer employment program of the Heart Institute provided summer jobs for 32 students. A study of this program after its completion indicated that the program was most successful in at least one respect: the students, by and large, felt that their working experience here had aided them in deciding on careers in science, and, in particular, interested them in future positions at NIH. Every effort was made to give these students jobs that were commensurate with their training and ability, to enable them to work as independently as possible, and to put them in close contact with responsible investigators.

The chief benefit to the NHI research program of this type of employment is that it provides a potential source for recruiting capable investigators - an advantage in days of technician shortage.

6. The Central Mail and Records Unit and the Travel Unit were combined under one administrative head in the Office of the Director. The Mail and Records Unit underwent extensive revisions which: reduced the number of subject classifications from 650 to 250; developed a plan for retiring inactive files routinely; resulted in better coordination with other Institute files.

The Travel Unit partially decentralized its services to compensate for increased participation in outside professional activities by NHI scientists. This was done by delegating to the Grants and Training Branch the handling of many of its own travel details and services. A system of logging was devised to maintain current information as to the status and ultimate disposition of both domestic and foreign travel documents. These logs also serve as a means for keeping a cumulative annual record of travel data.

The two major administrative problems encountered during the course of the year are problems that are not peculiar to the Heart Institute: space and personnel.

The Grants and Training Branch, which was for a time divided between Wilson House and T-6, has recently been moved into space in T-6 that is sufficient to house the entire branch.

However, the location of the Heart Information Center in Bethesda has been, and continues to be, a tremendous handicap to the staff of the Institute, creating communication problems and loss of time in travelling. Every

effort will be made in the coming months to obtain space for the Center on the reservation, in order to improve administrative and program relationship.

The major personnel problem is the recruitment of properly qualified technicians for laboratory work. Chemists at the GS-5 level are virtually unavailable, and there is every indication that this situation will continue.

This condition is reflected in the summer employment of students; several of the promising young scientists who were employed last summer by the Heart Institute have already indicated that financial considerations make it impossible for them to return.

For the future, two proposals are under consideration:

1. The Heart Information Center has in storage a number of exhibits that were prepared and used for various program purposes. It has been proposed that these exhibits be lent to high schools or colleges, as a means of stimulating interest in science and careers in scientific research. Details of this program have not yet been worked out, but it is felt that such an arrangement could serve the dual purpose of getting the exhibits out of storage, and stimulating scientific interest at this academic level.

2. As indicated above, the summer employment program for students was so successful that the Heart Institute proposes to program it for future summers. The suggestion has been made that there are some summer jobs that could be filled by high school graduates, or college undergraduates. This would mean reaching an additional group of potential scientists to stimulate their interest in scientific careers.

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CENTER FOR AGING RESEARCH
(organization)

The Center for Aging Research, established in September 1956, is responsible for the stimulation and coordination of research and training in the field of gerontology, and for the assembly and dissemination of data on gerontological studies. It works with all of the Institutes, but is administratively responsible to the National Heart Institute. The Center's principal responsibility is to work with the extramural program through contacts with individual investigators in university laboratories and research hospitals. The Center acts in a liaison capacity between NIH and programs having to do with gerontology in other federal agencies, universities, and national societies.

During this initial full year of activity, the staff of the Center for Aging Research has been concerned with program planning, contacts with the leaders in the field of gerontology, and participation in meetings and conferences. The staff members have attended the HEW and PHS conferences concerning development of the aging program within the Department and have cooperated with several of the Study Sections and National Advisory Councils in obtaining information and opinions on research in this field. The Center has cooperated closely with the Gerontological Society in a survey to delineate research needs in the field of gerontology; this survey is supported by a research grant from the National Heart Institute.

On the basis of the individual project reports of intramural research for the calendar year 1956, the staff of the Center identified and classified the research projects that were considered to be either primarily or secondarily related to research in aging. This compilation was duplicated and was made available to the Director of the National Institutes of Health and to the Institute Directors and Division Chiefs during the spring of 1957.

At the time the Center for Aging Research was established, it was the belief of the National Institutes of Health that the complex nature of research on aging justifies an experimental departure from the previous grant awarding practices. The National Institutes of Health, therefore, with the concurrence of all of the National Advisory Councils, planned to assist in the establishment of several large research centers operated by universities. During the winter and spring of 1957 the staff of the Center for Aging Research worked closely with investigators at Duke University in developing plans for the first such university-centered center in gerontology. As a result, a research program dedicated to a multidisciplinary study in gerontology was established at Duke University on September 1, 1957. This program is partially supported by a grant of just over $300,000 a year for five years. Upon the recommendation of their respective National Advisory Councils, the National Heart Institute and the National Institute of Mental Health are sharing equally in the grant.

At the present time the staff of the Center is working with several other university groups that are interested in developing similar large research programs in gerontology. One application has already been submitted for consideration by the National Advisory Heart Council at its meeting in February-March 1958, and others are in the process of development.

In furthering its objective of facilitating the dissemination of information concerning research and action programs in the field of aging, the Center worked with the United States representative of the Excerpta Medica Foundation. As a result, the National Heart Institute has made a five-year grant, in decreasing amounts, for the Excerpta Medica Foundation to establish a new Section in its series of abstract publications, on gerontology. This grant was approved at the November meeting of the National Advisory Heart Council, and the new Section on Gerontology will be started with an issue dated January 1958.

The staff of the Center for Aging Research, in cooperation with the staff of the Division of Biology and Medicine of the Atomic Energy Commission, has developed a plan for a series of conferences on the "Similarities and Differences Between Physiologic Aging and Radiologic Life Shortening". This series of conferences is being arranged by the American Institute of Biological Sciences through a grant of $19,550 from the National Heart Institute. The Atomic Energy Commission has made a similar award to AIBS. The purpose of these conferences is to bring presently known information to bear on the question of the relationship between physiologic aging and radiologic life shortening in order that the general problems of radiation-induced injury and ordinary senescense may be more effectively elucidated. The conferences will be held during the calendar year 1958 and it is hoped that a series of papers will be developed and published in book form that will establish a current base line of knowledge in this field.

The Director of the Center for Aging Research conferred with many outstanding European gerontologists in Europe in connection with the Fourth International Congress on Gerontology at Merano, Italy in July 1957. In addition, one or more members of the staff of the Center have attended meetings (and in most cases have participated in the programs) of the Gerontological Society, the American Geriatrics Society, the Committee on Aging of the American Medical Association, the American Hospital Association, the American Public Health Association, the American College of Surgeons, and the National Committee on the Aging. In his capacity as a member of the Board of Governors of the College of Surgeons, the Director of the Center proposed at the October meeting of the Board that the College undertake to facilitate the movement of well-trained surgeons from metropolitan areas to small towns during their latter years of practice, to provide a smooth transition for the surgeons from a busy metropolitan practice to eventual retirement, and to provide better surgical care in small towns. This proposal was well received by the Board of Governors.

Members of the staff of the Center have addressed a number of professional and non-professional groups during the year, speaking in each case on some aspect of the aging problem.

Two publications have resulted from the activity of the Center during 1957. These are:

Hunt, G. Halsey. Research in Aging. Medical Annals of the District of Columbia 26:186-187, April 1957.

Hunt, G. Halsey. The Key Role of the Physician in Geriatric Research. Geriatrics 12:679-681, November 1957.

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Heart Information Center

The Heart Information Center carries out a program of public and professional information on diseases of the heart and circulation, as specified in the National Heart Act. With reference to research in cardiovascular disease, the Center prepares and distributes information concerning research developments and advances, thus assisting the dissemination of new knowledge and helping to further the use of research findings. The Center also provides assistance to the research staff of the Heart Institute by furnishing consultation and services as needed in matters involving media of communication, including press relations, preparation of exhibits and other graphics, and clearance and printing procedures.

Broad objectives of the information program are to provide information on all aspects of heart disease; to promote increased application of present knowledge and research results; to assist prevention, case-finding, diagnosis, and treatment of heart and blood vessel diseases through lay education; and to keep individuals and organizations in the heart disease field informed as to current developments.

The diversified activities of the Heart Information Center include the preparation and distribution of information-education publications, such as leaflets, pamphlets, and other items; issuance of releases and providing assistance to the press, periodicals, and medical writers; preparation of reports, summaries of research findings, and other special materials; planning and development of exhibits, audio-visuals, graphics, and materials for broadcasting; maintenance of an inquiry and reference service; and cooperation with other agencies and organizations in information-education matters relating to cardiovascular diseases. Materials produced and activities conducted during 1957 included:

A booklet for the general public, "Cerebral Vascular Disease and Strokes", was produced and its widespread dissemination was planned and undertaken. As the first health education publication specifically devoted to this major cause of death and disability, the booklet fills a long-felt need for accurate, easily understood, up-to-date information on the subject. Demand for the booklet has been gratifying, necessitating immediate reprinting, with approximately 75,000 copies distributed, largely in response to requests, by the year's end. Another booklet, entitled "The Food You Eat and Heart Disease", prepared by the Center in cooperation with the Heart Disease Control Program, was completed and initial distribution made. The publication presents general information on diet and heart disease, currently a subject of much controversy and public interest.

Also issued was a booklet, "Highlights of Heart Progress", a compilation of items on program developments and accomplishments in research conducted and supported during 1956 by the National Heart Institute. This was printed in limited quantity principally for use at exhibits; it has been exceptionally well received and the Superintendent of Documents, GPO, has expressed interest in reprinting in order to have sales copies available. "A Living Pump", the simple graphic presentation of blood circulation which originally appeared in The Journal of Health, Physical Education and Recreation, was reprinted as a poster and is proving useful for meeting requests, particularly those from school children.

Work on a comprehensive publication, "The Heart in Health and Disease", continued. A tentative layout and illustrations for this primer on the normal circulatory system and the disorders that may affect it were developed during the year. Revision and up-dating of four basic leaflets, "Heart Disease", "Coronary Artery Disease", "Hypertension", and "Rheumatic Heart Disease" was also planned.

Press releases and services to writers continued to be useful means for dissemination of information on work of the Heart Institute and on noteworthy events relating to its program. Releases issued included nine on specific research accomplishments made by Institute scientists which were considered to be of general interest, and thirteen of a program nature, reporting grants, announcing appointments, or giving information on meetings or publications. In addition, several articles were prepared on request for use in syndicated feature columns under the byline of Institute staff members.

Many services were given newspapers, magazines, television, radio, and other media, through working directly with writers, reporters, and others. Background material was provided, special information obtained and furnished, or interviews arranged with Institute scientists and others in behalf of staff members of national publications, the working press, house organs, and free-lance writers. Among magazines serviced were Medical News, Scope, Life, Look, Time, Newsweek, U. S. News and World Report, Fortune, Readers Digest, Saturday Evening Post, Science Newsletter, Parade, and others.

Reports constituted another important area of activity, with numerous reports of regular or special nature being prepared in whole or in part. These included reports prepared each week of the year containing items of interest on selected cardiovascular research advances 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 over-all annual report of the Department; and weekly reports itemizing selected informational activities for the Office of Research Information and the PHS Information Service Office. Among special reports prepared or edited were a summary of Heart Institute conducted and supported research relating

to diseases affecting children, a listing of major research advances of recent years, one concerning informational operations prepared as part of a government-wide survey, a research progress report on cardiac catheterization, and others of an administrative nature, e.g., distribution of information issuances to regional offices and State health departments. Two issues of "Heart Memo", an administrative memorandum giving items of interest and developments relating to heart disease control, were written and issued in 1957.

Materials prepared or on which assistance was given in connection with budgetary and appropriations matters included a compilation of highlights of heart research accomplishments selected from papers published by Institute and grant-aided investigators in the medical literature within calendar year 1957, the opening statement for hearings on the Institute's appropriation, a summary of recent advances in heart disease research, descriptions of component areas of the Institute's research program, and other supporting information. During the year, draft or background materials were also prepared for a number of speeches, messages, and statements made in conjunction with various events and occasions.

The planning, overseeing production, and demonstration of exhibits was another area of considerable activity. The large exhibit, "National Heart Institute--A Medium of Service", continued to be useful for presenting information on the PHS heart program at health organization meetings. In 1957, it was demonstrated at the sessions of the New York Annual Health Conference, the Southern Branch of the American Public Health Association, the Georgia Public Health Association, the North Carolina Public Health Association, and on special request, at the convention of the General Federation of Women's Clubs. The technical exhibit for professional audiences, "Mechanism of Action of Reserpine and Chlorpromazine--An Hypothesis", was shown at the annual meeting of the American Medical Association.

A new exhibit completed during the year, "Here's Help for the Sodium-Restricted Patient", prepared in cooperation with the American Heart Association and the Heart Disease Control Program, BSS, was very well received and is proving of much value for educational purposes. It has been shown at annual meetings of the American Dietetic Association and the American Public Health Association. Another new exhibit, "Cerebral Vascular Disease and Strokes", was developed for use in promotion of the booklet on this subject; its first showings were at the scientific sessions and annual meeting of the American Heart Association and at the annual meeting of the National Society for Crippled Children and Adults.

The Heart Information Center participated extensively in connection with the program held at the National Institutes of Health commemorating the tercentenary of the death of William Harvey, the English

scientist who discovered the circulation of the blood. The program, at which addresses were given by noted Harvey scholars and cardiovascular scientists, presented knowledge of the circulatory system in the pre-Harvey era, Harvey's contributions, and developments since that time. A comprehensive exhibit, "William Harvey and the Circulation of the Blood", was produced jointly with the National Library of Medicine, for display in conjunction with the commemoration, and a catalog was prepared explaining the exhibit and the historical objects, many of great value, which were incorporated in it. The exhibit was shown at the National Institutes of Health for approximately one month, then at the National Library of Medicine for two months. Parts of the large exhibit will have subsequent showings at the Georgetown Medical School and at the Smithsonian Institution.

The Center also rendered services in connection with various other meetings, and assisted in the handling of visitors to the Heart Institute and NIH. Programs were planned and arrangements made for four Heart Seminars, given for American Heart Association and health department personnel during the year. Planned as extension training, the Seminars developed knowledge and familiarity with the program, activities and services of the NHI-PHS heart program among staff members of heart associations and health departments from many parts of the country. The Center further participated in meetings arranged for representatives from Department Regional Offices and State Health Departments.

The provision and maintenance of inquiry and reference services was a continuing function, with many requests for information from the general public, individuals in medical and health professions, and organizations, being received and answered during the year. In addition to mail, telephone, and in-person inquiries received directly by the Center, numerous inquiries were referred to it for appropriate handling by other units of the Institute, the Service and the Department, other government agencies, and members of Congress. The majority of requests for information received could be effectively serviced by forwarding available publications which contained basic general information relating to the more common diseases; others, however, required special compilation of information, involving consultation with scientists, preparation of reading and reference lists, or searching out pertinent data and material.

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BIOMETRICS RESEARCH SECTION

1957 marked the end of our participation in the Cooperative Study of Lipoproteins and Atherosclerosis with the publication of a report on serum lipid levels within the study group (Moore, Felix E., Gordon, Tavia, et al: Serum Lipid Levels in Normal Persons. Findings of a cooperative study of lipoproteins and atherosclerosis. Circulation 16: 227, 1957.) It also marked the end of the first four years of followup of the Framingham Heart Study population and a publication of the results of this study. (Moore, Felix E., et al: Coronary Heart Disease in the Framingham Study. Am. J. of Public Health. 47: 4, pt. 2. 4, 1957).

With the completion of this report Felix E. Moore resigned as Chief, Biometrics Research Section, effective July 1, 1957. A new chief has not yet been appointed and since July the remaining personnel have devoted their attentions almost exclusively to providing statistical services for the Framingham Heart Study Program. This has meant, for instance, a lapse in the mortality analysis program for the section. The last report in this area was Gordon, Tavia: Mortality Experience Among the Japanese in the United States, Hawaii and Japan. Public Health Reports. 72: 543, 1957.

The program for Framingham this year has involved:

1. Redesigning the IBM punch cards containing information from the first three rounds of examination, as well as the card deck containing information on smoking and alcohol consumption.

2. Designing and preparing coding forms for information from the fourth and fifth rounds of examination.

3. Arranging for a computer program for the dietary histories being collected from the fifth round of examination.

4. Planning and getting under way a program for the use and analysis of the data already collected. This has involved tabulation programs this year on blood pressure, uric acid levels and arteriosclerotic heart disease occurring de novo during the first six years of followup in the Framingham study population.

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Epidemiology Section, Framingham, Massachusetts

The Heart Disease Epidemiology Study has completed its eighth year of operation since beginning its study of a randomly selected population of adults in Framingham, Massachusetts early in 1950.

The objectives of this study remain essentially unchanged, i.e., to investigate and evaluate those factors which are believed to be significant in the development or the progression of hypertension, hypertensive vascular disease and arteriosclerotic heart disease.

In 1950, by random sampling method, 6,454 adults between the ages of 30 and 59 were selected for possible examination. Of these it was possible to obtain the cooperation of 4,494. An additional group of 740 volunteers in the same age range was added to the study. All cooperating persons were initially examined and were invited to have similar examinations at intervals of two years for a period which might extend to twenty years. During this time, it was planned to record all data of pertinent constitutional or conditioning factors.

Major findings which shows the status of examinations as of October 31, 1957, are found on the attached table.

As the major epidemiological investigations carried on by the Institute, this project should develop information leading to knowledge of the natural history of heart diseases, particularly atherosclerotic coronary disease, and hypertensive cardiovascular disease. Such knowledge would appear essential to the eventual development of practical control measures.

Accomplishment during the year:

Continued action toward insuring community cooperation in the project has been maintained through daily personal contacts with subjects and the local medical profession. In addition, several interviews have been provided to interested individuals leading to local and more wide-spread newspaper articles about the study. The effect of these in keeping up the interest of the community is believed to be considerable. The Medical Director has also spoken at several local Heart Association Meetings about the type of investigation being carried on.

Interest has been centered on a nutritional history now being obtained on a sub-sample of subjects undergoing their fifth examination. The method of

arriving at the qualitative and quantitative values used in Framingham was compared in a special study with those used elsewhere and it was demonstrated that essentially the same values were derived. Prosecution of the diet study will be continued with periodic re-evaluation.

The remainder of the examination procedure has been carried out essentially as before.

In June 1957, a two-day meeting was held under the auspices of the Heart Disease Epidemiology Study to which the major groups in the United States carrying out longitudinal cardiovascular studies were invited. Problems in any way related to conducting such studies were discussed and several proposals for cooperation between the groups were made. Some of these, i.e., a serum pool for cholesterol standardization, were considered suitable for further action. It is believed this meeting was of considerable value and that similar gatherings should be considered in the future.

Publications from this project during the calendar year 1957 are as follows:

1. "Coronary Heart Disease in the Framingham Study". T.R. Dawber, M.D.; F. E. Moore, F.A.P.H.A.; and George V. Mann, M.D. Journal of Public Health, Vol. 47, No. 4, April 1957.

2. "An Epidemiology Study of Heart Disease - The Framingham Study". T.R. Dawber, M.D. and W. B. Kannel, M. D. To be published in the January 1958 issue of NUTRITION REVIEWS.

3. "The Electrocardiogram in Neurocirculatory Asthenia. (Anxiety, Neurosis or Neruasthenia): A Study of 203 Neurocirculatory Asthenia Patients and 757 Healthy Controls in the Framingham Study". T.R. Dawber, M.D., F.A.C.P. W. B. Kannel, M.D., and Mandel E. Cohen, M.D. Accepted for publication by the ANNALS OF INTERNAL MEDICINE.

4. "Essential Fatty Acids and Atherosclerosis" George V. Mann, M.D. A.M.A. Archive of Internal Medicine, July 1957, Vol. 100, pp. 77-84.

Problems encountered: The difficulty of standardizing laboratory procedures e.g. blood cholesterol, which cannot be compared to a fixed standard has been of considerable concern. As a result of a meeting of the various groups in this country interested in longitudinal cardiovascular studies held under our auspices in June 1957, a plan was devised to set up a serum pool, samples of which could be forwarded to cooperating laboratories. Plans are now underway at the Communicable Disease Center, Atlanta, Georgia to carry out the project. The Staff of the Heart Disease Epidemiology Study has agreed to consult with the Communicable Disease Center group and help them in any way possible to perform this very needful service.

Future Objectives: Preliminary meetings have been held regarding studies to be carried out in the sixth examination of the population beginning Octobber 1958. A study of stress and anxiety has been planned. Methodology of such a study is now in the planning stage.

A pilot project to determine estrogens and androgens on a small number of male subjects has been planned with the help of the Worcester Foundation for Experimental Biology. If this study is successful further plans to carry out determinations on a larger group will be considered.

There have been discussions regarding the advisability of extending the study population to include a new group of 30 year old persons. The desirability of this will receive careful consideration and if deemed advisable a proposal to this effect will be made early in 1958.

An agreement was reached with the Atomic Bomb Casualty Commission to carry on certain comparative studies between their study population in Hiroshima, Japan, and ours in Framingham, Massachusetts. Exchange of serum specimens for standardization of cholesterol determinations has been started and preparations made in Hiroshima to develop comparable studies to our own.

STATUS OF EXAMINATIONS AS OF OCTOBER 31, 1957

Number Selected	6510
Number Examined	4469 = 68.6%
Plus SX	740
	5209

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	3,758	3,060	698
V	1,194	784	410

Number Not Examined, Examinations II and III (a)

II	418	418	0
	45	45	- Deceased
	159	159	- Examined later round.
	214	214	- Lost
III	553		
	101	91	10 Deceased
	108	101	7 Examined later round.
	351	340	11 Lost

(a) As of October 31, 1957.

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GRANTS AND TRAINING BRANCH

Research Grants:

An appropriation of $19,364,000 was made for research projects in fiscal year, 1958. As of December 1, 1957, 1,301 research grant applications have been recommended favorably by the National Advisory Heart Council in the amount of $19,022,927. At the meeting of the National Advisory Heart Council, to be held February 27-28, March 1, 1958, it is probable that more than 330 competing applications will be considered.

Training Grants:

An appropriation of $5,135,000 was made in fiscal year 1958 for research and clinical training grants at the undergraduate and graduate levels and for direct traineeships. As of December 1, 1957, training grants have been awarded in the amount of $4,805,816 to 198 institutions. A total of 23 direct traineeships in the amount of $116,123 have been awarded during the fiscal year. It is probable that approximately 35 training grant applications will be considered at the February 1958 meeting of the National Advisory Heart Council.

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II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1957 DATE: August 1, 1957

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Program

3. SECTION
Operational Research

4. DIVISION PROJECT NUMBER
SHS-HD-6

5. PROJECT TITLE
Job requirements and work capacity of persons with cardiovascular disease.

6. INVESTIGATORS
Dr. John Wuest
Dr. David Geddes
Mr. Philip Enterline

7. LOCATION OF PROJECT
Los Angeles, California

8. STATUS
Completed

9. PARTICIPATING ORGANIZATIONS
Cedars of Lebanon Hospital - facilities
Lockheed Aircraft Company - personnel and facilities

10. PRINCIPAL RESULTS
The final results of this project were described in the progress report dated July 30, 1956. During the past year equipment was loaned to the Department of Physiology, Brigham Young University, Provo, Utah. Under the direction of Dr. David Geddes, further work will be done towards validating special equipment developed as a result of this project. Subsequent reports will be issued by Brigham Young University. Active participation on the part of the Heart Disease Control Program has ceased.

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II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1957 DATE: August 1, 1957

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Program

3. SECTION
Operational Research

4. DIVISION PROJECT NUMBER
SHS-HD-7

5. PROJECT TITLE
Study of the value of heart disease case finding by X-ray.

6. INVESTIGATORS
Dr. William H. Stewart
Dr. Bernard Kordan
Mr. Philip Enterline

7. LOCATION OF PROJECT
Los Angeles, California
and Dallas, Texas

8. STATUS
Completed

9. PARTICIPATING ORGANIZATIONS
California State Health Department - facilities
Los Angeles City and County Health Department - facilities
Chest X-ray Survey Foundation - personnel, facilities and funds
Texas State Health Department - personnel and funds
Dallas City Health Department - personnel and funds
Dallas Tuberculosis Association - facilities

10. PRINCIPAL RESULTS

Analysis of data was completed during the year and a paper presented at the annual meeting of the National Tuberculosis Association in May 1957. Subsequently, a paper was prepared for publication in the American Review of Tuberculosis. An abstract of this paper also appeared in the proceedings of the annual meeting of the National Tuberculosis Association.

The study revealed that cardiovascular disease suspects picked up on chest X-ray surveys receive definite benefits as the result of discovery. During the three year period following chest X-ray programs, the medical and nursing care given these persons reduced their mortality rate by an estimated 15%. The study also revealed a considerably greater reduction in mortality rates for tuberculosis

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10. PRINCIPAL RESULTS - Continued SHS-HD-7

suspects; suggests that the death rates for tuberculosis underestimate the true importance of this disease as a cause of death; resulted in an estimate of a 1% reduction in mortality in total populations participating in mass photofluorographic surveys during a three year period following such participation as a result of medical and nursing care given those detected with disease. The study has proven particularly valuable in deciding upon the merits of radiographic surveys in relation to supposed radiation hazards involved.

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II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1957 DATE: August 1, 1957

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Program

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. William H. Stewart
Mr. Donald Loveland
Mr. Philip Enterline

7. LOCATION OF PROJECT
Los Angeles, California

8. STATUS
Continuing

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 paper on coronary heart disease among civil service employees was presented at the meeting of the American Public Health Association in September 1956 and subsequently published in the April issue of Public Health Reports. The study revealed that 2.7% of the adult males initially had coronary heart disease. The average annual incidence of coronary disease in males with normal heart on entry was 0.1% under 40 years of age, 0.8% for 40-54 years and 2.9% for 55-70 years of age.

During the year a draft manuscript was produced on a study using the civil service employees physical examination data and relating to the ability of chest photofluorograms to detect heart disease. This paper reveals that whereas it was initially reported that only 4% of the heart disease in this group of Los Angeles civil service employees could be detected by chest X-ray, a second reading of the same films by the same reader, but with attention paid to cardiovascular

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10. PRINCIPAL RESULTS - Continued SHS-HD-8

abnormalities, resulted in the identification of 50% of the existing definite heart disease. A third reading of the same series of films by a trained radiologist resulted in the identification of 71% of the existing definite heart disease. This study serves to demonstrate the potentiality of chest X-ray as a case finding procedure for cardiovascular disease, if careful attention is paid to abnormal cardiovascular shadows.

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II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1957 DATE: August 1, 1957

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
Continuing

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

10. PRINCIPAL RESULTS
Technical improvements have been made in the heart sound recorder equipment, and a study made of 100 institutionalized children. Analyses of this limited amount of work indicates that the heart sound recorder is just as sensitive and specific as other tests for the detection of heart disease in children. An additional study is underway in another institution of 1000 children and further exploration is being made into problems concerned with the development of listening criteria.

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II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1957 DATE: August 1, 1957

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
Coronary heart disease in vegetarians

6. INVESTIGATORS
Dr. William J. Zukel
Dr. David C. Miller
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

Hospital records have been studied to determine the numbers of Seventh Day Adventists and other religious groups admitted to the hospital. There are an estimated 200 males, 35 to 70 years of age, admitted annually who are Seventh Day Adventists. A plan for selection of controls for these has been developed along with a dietary interview to be administered both groups. Blood cholesterols and dietary information will be obtained from all newly admitted Adventists and a like number of controls starting about September 1, 1957.

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II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1957 DATE: August 1, 1957

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
Dr. Herbert S. Caron

7. LOCATION OF PROJECT
Washington, D. C.

8. STATUS
Continuing

9. PARTICIPATING ORGANIZATIONS
D. C. General Hospital - facilities
Georgetown University Hospital - facilities

10. PRINCIPAL RESULTS
An interview form was developed and intensively used on a group of 25 patients, data was assessed for its relevance to hypotheses developed during the previous year. Those hypotheses that received tentative confirmation from the study have been isolated for further investigation to measure "excessive disability" and the reasons for favorable response to rehabilitation techniques in this and a new group of coronary patients. A 20-page paper and a summary have been prepared for publication.

Department of Health, Education, and Welfare
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NH1-13

II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1957 DATE: August 1, 1957

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Program

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
Tables are completed. A meeting has been arranged with Dr. Graves of the Tennessee Health Department and his staff regarding the interpretation of the data and the preparation of the report.

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II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1957 DATE: August 1, 1957

1. DIVISION
Special Health Services

2. BRANCH
Heart Disease Control Program

3. SECTION
Operational 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
Preliminary results show very low serum cholesterol levels, consistent with the hypothesis that population groups having a low percent calories from fat have a low serum cholesterol level. Collateral data such as height, weight, skinfold measures of obesity, blood pressure, and phospholipids were also analyzed to relate to serum cholesterol levels. A report is now being prepared by Dr. Newman of Smithsonian Institute. Our phase of the project has been completed.

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II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1957 DATE: August 1, 1957

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. John Summerskill, American College Health Association
Mr. Herbert I. Sauer

7. LOCATION OF PROJECT
Washington, D. C.

8. STATUS
Continuing

9. PARTICIPATING ORGANIZATIONS
American College Health Association - Student Health Services of 135 participating colleges - personnel and facilities

10. PRINCIPAL RESULTS
About 70,000 examinations have thus far been reported for the school year 1956-1957. An analysis of the first 54,000 revealed that 2.4% had definite or possible rheumatic heart disease or history of rheumatic fever. The percentage of these on anti-streptococcal prophylaxis range from 2% for students with a questionable history of rheumatic fever, to 22% for those with definite rheumatic heart disease. A complete report of the first year's experience with this study will be prepared this fall. A paper on the preliminary results of this project was presented at the annual meeting of the American College Health Association in April, 1957. The results of the first year's examination have been distributed to participating colleges and universities.

Department of Health, Education, and Welfare
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II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1957 DATE: August 1, 1957

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
Mrs. Eloise Worden

7. LOCATION OF PROJECT
Boston, Massachusetts

8. STATUS
Continuing

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

10. PRINCIPAL RESULTS
Exploratory period has been completed. A meeting is planned for early fall to design the second phase of the Study, which will test selected questions with a larger patient sample. It is expected that the exploratory material will also yield a list of questions which can be considered for further study elsewhere.

Department of Health, Education, and Welfare
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II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1957 DATE: August 1, 1957

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

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 Devil's Lake Medical Societies, North Dakota

10. PRINCIPAL RESULTS

Reporting of coronary disease started on September 1, 1956. A census involving a ten percent probability sample of the population was taken in November, 1956. Starting on January 1, 1957, a control matched for age was selected from the census material for each new case of coronary disease reported and controls and cases were given a detailed interview with emphasis on dietary information. All census records have been coded and punch cards prepared and a population estimate is now available for the area. Analysis of detailed interviews of a probability sample of males 35 years of age and over has begun. Reporting of coronary disease will cease on December 1, 1957.

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II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1957 DATE: August 1, 1957

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 OF PROJECT
Hagerstown, Maryland

8. STATUS
Continuing

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

10. PRINCIPAL RESULTS
Abstracts of 2400 records on school physical examinations have been completed.

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II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1957 DATE: August 1, 1957

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. Tyroler
   Mr. Sidney Abraham

7. LOCATION OF PROJECT
   Asheville, North Carolina

8. STATUS
   Continuing

9. PARTICIPATING ORGANIZATIONS
   Asheville Research Foundation

10. PRINCIPAL RESULTS

A letter, proposing the study, was sent to Asheville Research Foundation, on May 28, 1957. On June 10, Dr. Tyroler, Director, replied indicating an interest of exploring with us the study project. A meeting was held with Dr. Tyroler on July 29 in Asheville. It was agreed that the first step in developing the study was for Dr. Tyroler to send us statistical data showing the age breakdown of white males in the three plants.

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II. RESEARCH PROJECT REPORT OF PROGRESS

FISCAL YEAR: 1957 DATE: August 1, 1957

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 Variation by Country and State of Birth.

6. INVESTIGATORS
Mr. Herbert I. Sauer
Miss Margaret Evans

7. LOCATION OF PROJECT
Washington, D. C.

8. STATUS
Continuing

9. PARTICIPATING ORGANIZATIONS
National Office of Vital Statistics

10. PRINCIPAL RESULTS

Preliminary comparisons indicate that, regardless of the combinations of major cardiovascular diseases used, death rates are substantially higher for the United States than for countries such as Sweden or Italy.

Tabulation plans are being completed in a way to utilize both work done in the past, and presently available resources, particularly mechanized procedures for tabulating data. A substantial portion of library research has been completed.

A number of desirable comparisons have been rejected because comparable data were not available; areas and groups have been selected in such a way that (a) population and mortality can be obtained for the same groups, thereby making it possible to compute rates, and (b) data are available for specified European countries for comparison with the groups living in the Middle Atlantic States who were born in those countries.

One illustration of the contrast in rates: For the age group 45 to 64, the cardiovascular renal death rate in South Carolina was higher in 1950 than the death rate from all causes of death for 15 other States.

8-28-57

Form No. ORP-2
Oct. 1957

PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
Laboratory of Chemistry of Natural Products

Estimated Obligations for FY 1958

Total:	$333,979
Direct:	$240,141
Reimbursements:	93,838

Form No. ORP-2
Oct. 1957

Serial No. NHI-21
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Structure of Amaryllidaceae Alkaloids

Principal Investigators: W. C. Wildman, Ph.D.
Shojiro Uyeo, Ph.D.

Other Investigators: Elizabeth J. Kielar, A.B.
Antoinette A. Valesquez, A.B.

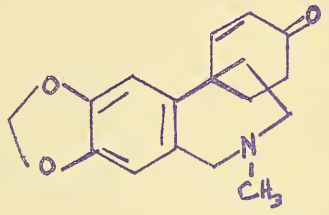
Cooperating Units: None

Man Years (Calendar year 1957):
Total: 2.75
Professional: 1.25
Other: 1.50

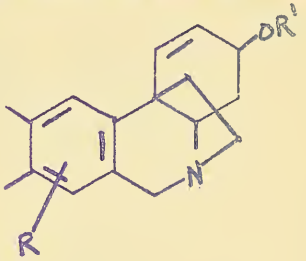
Patient Days (Calendar year 1957):
None

Project Description:

Conclusive chemical evidence for the structure of crinine and several related alkaloids has been obtained through the conversion of oxocrinine to an optically inactive dienone (I). Of the three possible structures for crinine, only II (R,R'H) is compatible with the observed inactivity of the methine.

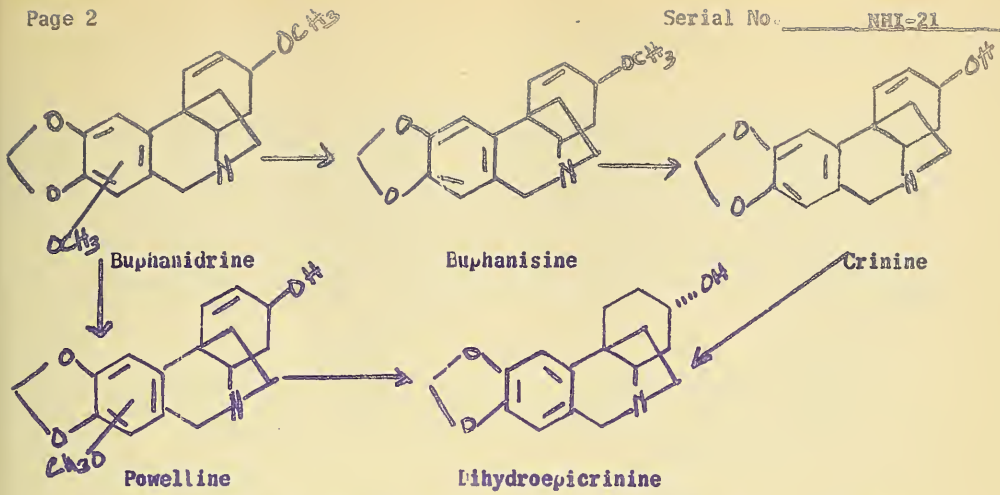


I

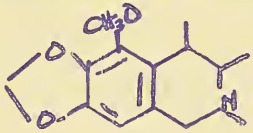


II

The use of sodium and amyl alcohol in the reductive cleavage of aromatic methoxyl groups has shown that buphanisine is Ar-demethoxy buphanidrine and powelline is Ar-methoxy crinine. These alkaloids have been interrelated in the following manner:

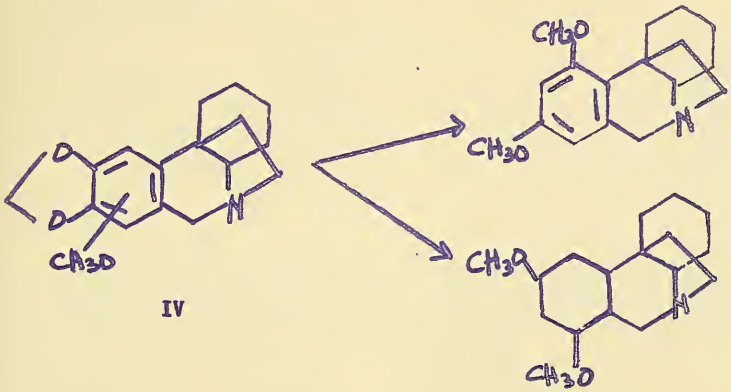


From biogenetic speculations, it would seem that the unplaced aromatic methoxyl group of the alkaloids containing the methylenedioxymethoxyphenyl group always should be located as shown in (III). A project to ascertain the correctness of this suggestion is in progress.



III

The Birch reduction of powellane (IV) has afforded a methoxyphenol. Methylation of this compound with diazomethane has given a dimethoxy derivative, which must be either Va or Vb. Syntheses of both Va and Vb are in progress.

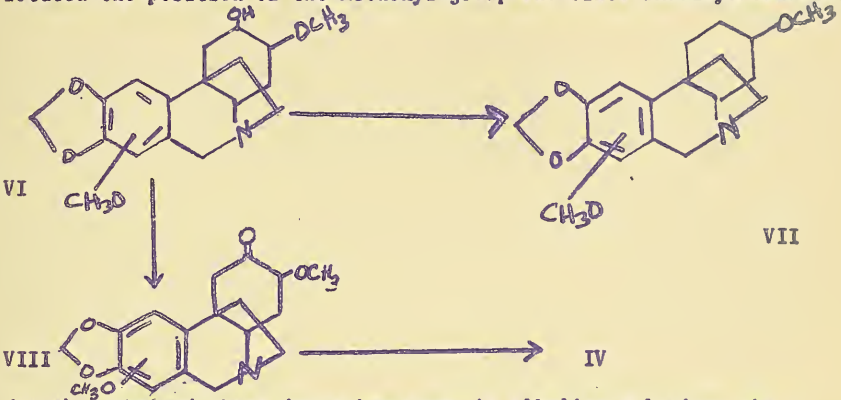


IV

Va

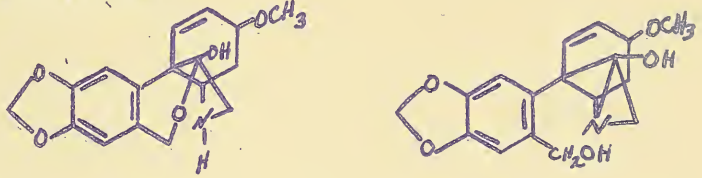
Vb

The structure (VI) has been proposed for dihydroundulatine. This formula is based on the observation that oxodihydroundulatine has been converted to powellane (IV). The carbonyl group has been placed in the ring C by spectral studies and the methoxyl group is adjacent to it since the latter is reductively cleaved by zinc and acetic acid. Tosylation of dihydroundulatine (VI) and reduction of the product with lithium aluminum hydride gave dihydrobuphanidrine (VII). This fact located the position of the methoxyl group and since oxodihydroundulatine



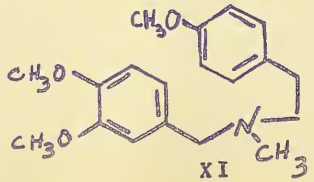
(VIII) exchanged three deuterium atoms in alkaline solution, the hydroxyl group of VI (and the carbonyl group of VIII) must be in the 2-position.

The structure (IX) has been proposed for haemanthidine on the basis of its conversion to tazettine by reductive methylation. From chemical and spectral studies, it has been shown that structure (IX) is not correct at present. Haemanthidine appears to be best represented by X, which is unique among alkaloids in that it contains an ethylene-imine ring system.



IX X

With the finding that the alkaloid belladine (XI) has anti-fibrillatory activity, representative alkaloids of the Amaryllidaceae have been submitted for further testing in this respect.



XI

Direction of Current Research: The isolation and characterization of alkaloids and other physiologically active constituents of selected plant familiar will be continued.

Part B included: Yes [XX] No [ ]

Serial No. NHI-21

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Invited lecturer: The Gordon Research Conference on Steroids and Natural Products August 5-9, 1957.
Ninth Annual Conference on Natural Products, Fredericton, N.B. October 23-25, 1957.

Briggs, L. H., Colebrook, L. D., Fales, H. M., and Wildman, W. C., The Infrared Spectrum of Methylenedioxy and Methoxy Groups, Anal. Chem. 29, 904 (1957).

Warnhoff, E. W. and Wildman, W. C., Alkaloids of the Amaryllidaceae X, The Structure of Caranine, J. Amer. Chem. Soc., 79, 2192 (1957).

Form No. ORP-2
Oct. 1957

Serial No. NHI-22
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Synthesis and Degradation of the Amaryllidaceae Alkaloids. Alkaloids of Tecoma stans.

Principal Investigator: Henry M. Fales, Ph.D.

Other Investigators: None

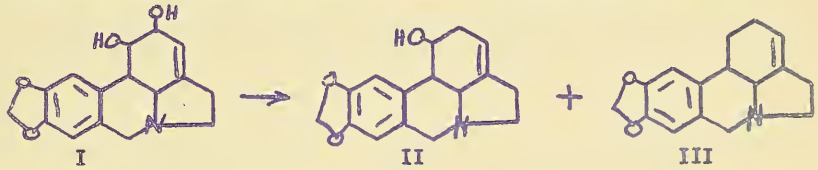
Cooperating Units: None

Man Years (Calendar year 1957):
Total: 1
Professional: 1
Other:

Patient Days (Calendar year 1957):
None

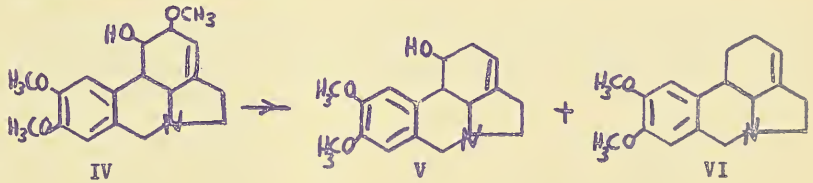
Project Description:

Methods have been developed for interrelating the Amaryllidaceae alkaloids. Thus, by the application of sodium and amyl alcohol, lycorine I has been converted to caranine II and lycorene III



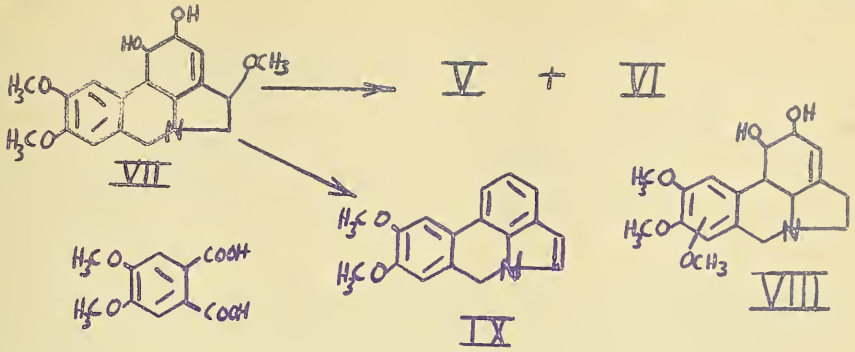
I II III

Similiarly galanthine IV has been converted to pluviine V and methylpseudolycorene VI.



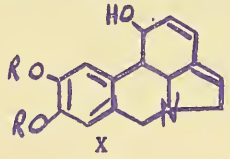
IV V VI

The structure of narcissidine is now represented as VII instead of VIII as suggested earlier. It furnished metahemipinic acid on



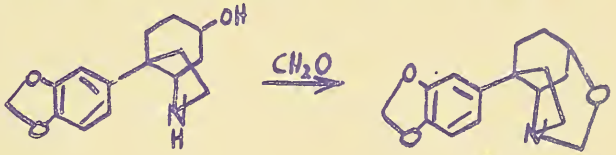
oxidation and V and VI on sodium and amyl alcohol reduction, while on treatment with powdered glass an indole, probably IX, resulted.

The intermediate in the above reactions of sodium and amyl alcohol has been isolated and shown to possess the diene structure X. This represents the first example, to the investigator's knowledge, of 1,4-base-catalyzed elimination.



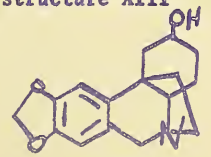
X

The synthesis of dihydrocrinine mentioned earlier has failed in the last step due to the formation of a cyclic ether XI

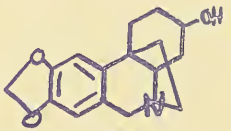


XI

An attempt is being made to complete the synthesis by another route. Other synthetic approaches leading to structure XII for dihydrocrinine have been abandoned since the natural base has been shown to possess the structure XIII



XII



XIII

Progress has been made on the structure of natalensine, now thought to possess structure XIV

XIV XV

It is thus an hydroxylated crinine derivative. Oxidation with pyridine-chromic acid produces the 5-membered ketone derivative XV which exhibits peculiar spectral properties. Reduction with sodium borohydride converts this ketone to an alcohol thought to be an epimer of natalensine.

No further work has been done on tecomanine, but samples of the pure alkaloid and the crude extracts have been sent to the pharmacological laboratories for testing as an antidiabetic agent.

Direction of Current Research: The structures of narcissidine and natalensine are being investigated further. An attempt will be made to complete the synthesis of crinine. Further studies of infrared absorption in the 2.5-4.0 μ region are in progress.

Part B included Yes [XX] No [ ]

Serial No. NHI-22

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Briggs, L. H., Colebrook, L. D., Fales, H. M., and Wildman, W. C. The Infrared Spectrum of Methylenedioxy and Methoxy Groups. Anal. Chem. 29, 904 (1957).

Form No. ORP-2
Oct. 1957

Serial No. NHI-23
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Alkaloids of Ormosia sp.

Principal Investigator: H. A. Lloyd, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years (Calendar year 1957):
Total:
Professional:
Other:

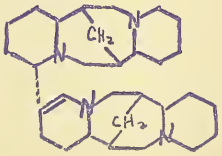
Patient Days (Calendar year 1957):
None

Project Description:

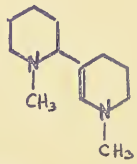
Seeds of Ormosia panamensis, Ormosia jamacensis and other Ormosia species were extracted. The extracts were fractionated by crystallization and by alumina chromatography. Ormosinine (OP I), ormosanine (OP II), panamine (OP III), methylcytisine, and several other new alkaloids were isolated in this manner.

The molecular formula $C_{20}H_{33}N_2$ for OP I and OP II was confirmed by the preparation of new derivatives: methiodide and hydriodide. There was not enough OP II to do any further work with this alkaloid.

The presence of a double bond in OP I was shown by catalytic hydrogenation to be a dihydro derivative. The position of the double bond relative to two of the nitrogens was tentatively assigned as $\alpha\beta$ to one nitrogen and $\beta\gamma$ to the other [N-C-C=C-N] by comparison of the UV spectra in acid of OP I, diplospartyrine (A) and dehydro N,N'-dimethyl-α,β-dipiperidyl (B)



(A)



(B)

New derivatives of panamine (OP III) were prepared and confirmed the $C_{20}H_{33}N_3$ formula. The analyses of N-methylpanamine and its derivatives (perchlorate, picrate, methiodide) were also in accord with this formula. No further work was done on the air oxidation product of panamine. Degradative studies starting with N-methylpanamine methiodide will be attempted when enough material is accumulated.

A new alkaloid OP IV, m.p., 80-81°C, was isolated in traces from Ormosia panamensis and in larger quantities from Ormosia jamacensis. This alkaloid differs from I, II and III in that it is oxygenated and contains only two nitrogens. Analyses of the alkaloid, its hydrochloride, picrate, and methiodide are in agreement with the formula $C_{14}H_{22}ON_2$ for OP IV. The infrared spectrum shows that one nitrogen is secondary (confirmed by positive Simon test); the other nitrogen and the oxygen are present in a lactam function. The lactam group can be reduced at room temperature and pressure with Adams' catalyst in hydrochloric acid solution. The IR spectrum also indicates the presence of a double bond, probably in a terminal methylene group (bands at 3.26, 10.2 and 10.9 μ). OP IV was reduced to a dihydro derivative with Pt in acetic acid solution. A hydrochloride of dihydro OP IV was prepared. Further evidence of the terminal methylene group was obtained by showing that formaldehyde is formed in the oxidation of the double bond with periodic acid and a trace of permanganate.

Another new oxygenated alkaloid was isolated in small amounts from the seeds of Ormosia jamacensis. OP V, m.p. 184-185°, gives a positive Simon test for secondary amines; its IR spectrum shows it contains a lactam group (piperidone or quinolizidone). There is no evidence of a double bond in the IR spectrum.

Methylcytisine was extracted in high yield from Ormosia stipitata, a Panamanian species. Its N-oxide was prepared in view of studying its rearrangement. However all attempts to rearrange the N-oxide by the usual procedures were unsuccessful, probably because of steric requirements.

Direction of Current Research: The investigation of the alkaloids of Ormosia sp. will be continued.

Part B included Yes [XX] NO [ ]

Serial No. NHI-23

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Lloyd, H. A. and Horning, E. C. Horning, Alkaloids of *Ormosia panamensis* Benth. and Related Species. J. Am. Chem. Soc., 79, 0000 (1957).

Form No. ORP-2
Oct. 1957

Serial No. NHI-24
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Alkaloids of Annona Sp. (Anonaceae)

Principal Investigator: Perola Zaltzman (Visiting Scientist)

Other Investigators: None

Cooperating Units: None

Man Years (Calendar year 1957):
Total: 0.5
Professional: 0.5
Other:

Patient Days (Calendar year 1957):
None

Project Description:

This project was started in October, 1956 and interrupted in May, 1957, awaiting a new shipment of plant material. Work resumed in December.

Anona Cherimolia had shown a marked hypotensive action in dog, and the objective of this study was to isolate and identify the alkaloids present in the plant.

Two alkaloids which were isolated and characterized have not been reported previously:

Alkaloid	Formula	Crystals	OCH_3	NCH_3	I.R.	U.V. (mμ)	Fluor. (mμ)
X	$C_{17}H_9O_3N$	Yellow Needles m.p. 272° (dec.)	None	None	>CO	248 269 309 415	Act 450 ↓ fluor. 520
Y	$C_{19}H_{21}O_4N$	White decomposes (180°)	2	1	OH	270 305	Act 320 fluor. 415

The U.V. spectrum of Y suggested an aporphine type alkaloid.

Direction of Current Research: The project will be resumed to elucidate the structure of X and Y and isolate some of the other alkaloids

of both A. Cherimolia (Costa Rica) and A. reticulata (Philippines), as well as to proceed with further pharmacological tests.

Part E included: Yes ☐ No ☒ XX

Form No. ORP-2
Oct. 1957

Serial No. NHI-25
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Cyclization of β-aryl ethylamines and Related Amino Acids.

Principal Investigator: Perola Zaltzman (Visiting Scientist)

Other Investigators: None

Cooperating Units: None

Man Years (Calendar year 1957):
Total: 0.5
Professional: 0.5
Other:

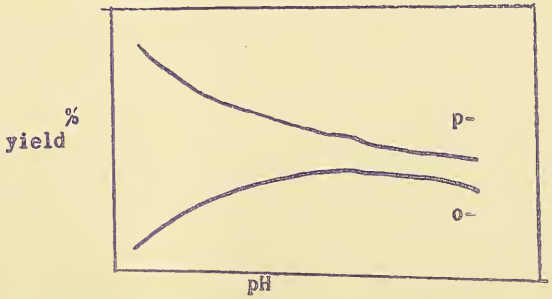
Patient Days (Calendar year 1957):
None

Project Description:

This project was started in June 1957 and should be terminated in early November.

The objective of this study was to investigate the conditions of pH dependency of the condensation reaction of m-hydroxy and 3,4-dihydroxyphenethylamines and related amino acids with formaldehyde, and then extend it to a possible pattern of biosynthesis of isoquinoline alkaloids in plants.

Paper chromatography showed that at room temperature there are two products as result of ortho and para cyclization, with a consistent pH dependency. When the pH rises from 4 to 7, the yield of para compound decreases and that of ortho compound increases, so that in the case of m-tyrosine, for example, the yield is almost 50% of each isomer at pH 7.



The mechanism of the reaction was not established but there are indications of a common intermediate that cyclizes at either activated position depending on the pH of the medium.

The identities of the o- and p-substituted cyclization products was established by independent synthesis of the p-isomers.

Direction of Current Research: This project is nearing completion.

Part B included: Yes ☐ No ☒

Form No. ORP-2
Oct. 1957

Serial No. NHI-26
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Isolation and Characterization of a Non-dialyzable Vasodilator Present in Human and Dog Urine.

Principal Investigator: Jack V. Pierce, Ph.D.

Other Investigators: None

Cooperating Units: Dr. Sarnoff, Dr. Sussman, L. C. Sarnoff (Laboratory Cardiovascular Physiology)

Man Years (Calendar year 1957)
Total: .75
Professional: .75
Other:

Patient Days (Calendar year 1957):
None

Project Description:

Dr. Sarnoff and coworkers have found that normal human and dog urine cause a transitory vasodilation in the femoral and coronary arterial beds of the dog. They worked out a highly sensitive and reproducible assay method which has made possible the development of methods for the purification of the vasodilator.

Early studies provided the following facts: (1) The vasodilator activity in human and dog urine can be separated into dialyzable and nondialyzable components. (2) The nondialyzable component (s) accounts for about 80 to 90 percent of the total activity. (3) The dialyzable component is reversibly and nondialyzable component is irreversibly absorbed by charcoal. (4) The nondialyzable vasodilator (NDV) can be precipitated from urine by addition of uranyl acetate or of lead acetate and the activity can be recovered from the precipitate by elution with diammonium phosphate (DAP). (5) The activity in the dialyzed lead acetate preparation could be adsorbed quantitatively on a column of acid-washed alumina and eluted in high yield by DAP. (6) NDV activity is destroyed by incubation with human serum for 0.5 hours at 37° C. (7) About 50 to 80 percent of the activity can be precipitated with 80 percent saturated ammonium sulfate.

By passing clarified urine at pH 4.0 and at 2° C over a column of acid-washed alumina or of the ion exchange resin XE-64 (buffered at pH 4.0), all the activity is adsorbed and can be quantitatively recovered by elution of the column with sodium phosphate buffer, pH 5.5 or with DAP solution. The purification is about 500-fold on a weight

basis and about 50-fold on the basis of protein content (biuret method of Laury et al.). The salt-free DAP eluate from an alumina column on 18 liters of urine is about 25 percent as potent as "Padutin", a commercial callicrein preparation from hog pancreas.

The facts listed in the first paragraph above also apply to callicrein, discovered by Frey and Kraut in 1926. No differences have yet been found between NDV and "Padutin".

Direction of Current Research: Further work on the purification of the urinary vasodilator from the alumina column is being pursued: chromatography on XE-64 and on the cellulose ion exchanger DEAE-SF.

A column chromatographic procedure suitable for use in the assay of human urine for callicrein content has been developed. Applications are being explored by Dr. Sarnoff and his colleagues.

Part B included: Yes ☐ No ☒

Form No. ORP-2
Oct. 1957

Serial No. PAI-27
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on Alkaloids. To discover new Alkaloids of potential therapeutic value and to elucidate their structures.

Principal Investigators: S. M. Goodwin, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years (Calendar year 1957):
Total: 1.0
Professional: 1.0
Other:

Patient Days (Calendar year 1957):
None

Project Description:

A. Ocotea leucoxylon.

(1) The methyl ether of Alkaloid C which was described previously was converted to the methiodide, m.p. 214-217° which was subjected to the Hofmann degradation. The methine, $C_{22}H_{23}O_5N$, m.p. 122-123°, obtained in good yield, was optically inactive and retained the three methoxy groups of the precursor. Second stage Hofmann degradation on the methine methiodide afforded a nitrogen-free material $C_{20}H_{18}O_5$, m.p. 166-168°, having three methoxyl groups. Dicentrine, an alkaloid of known structure found in O. leucoxylon, was subjected to the two-stage Hofmann in order to obtain its methine and vinylphenanthrene for ultraviolet spectral comparisons. Alkaloid C methyl ether methine and the nitrogen-free compound have UV spectra essentially identical with the corresponding Hofmann products in the dicentrine series. This evidence further established that Alkaloid C is of the aporphine class and that the fifth oxygen is situated in the aromatic portion of the molecule.

(2) Similarly, the crystalline methiodide of the oily Alkaloid, A, afforded an optically inactive methine, $C_{23}H_{27}O_6N$, m.p. 115-117°, retaining the four methoxyl groups of A and having an ultraviolet spectrum practically the same as dicentrine methine. Alkaloid A is, therefore, undoubtedly of the aporphine class having six of the seven available aromatic positions occupied by oxygen substituents.

(3) In repeating the isolation of the alkaloids, using an improved scheme which had been developed, the fraction in which Alkaloid A occurred afforded a crystalline alkaloid, $C_{22}H_{25}O_6N$, m.p. 54-56°, $[\alpha]_D$ + 52°, having 4-methoxyl and one methylimino groups. The paper chromatography, ultraviolet and infrared spectra suggested that this material was, at last, Alkaloid A crystalline. The methiodide, m.p. 227-230° (dec.), $[\alpha]_D$ + 33°, corresponded with that of the oil although some fractions of the oil afforded a methiodide of $[\alpha]_D$ + 41°. It is now felt that the oil, was a mixture consisting mainly of material corresponding to the crystalline alkaloid and a small amount of a strongly rotating, isomeric compound; at least, the oil was not completely homogeneous by paper chromatography. Further correspondence between the crystalline alkaloid and the oil was furnished by the conversion of the former to its methine, $C_{23}H_{27}O_6N$, m.p. 119-120°, which did not depress the melting point of the methine derived from the oil.

(4) Fairly large amounds of Alkaloid C methyl ether, dicentrine tartrate, and Alkaloid A hydrochloride were prepared for pharmacological studies by Drs. Moran and Pfeiffer of Emory University.

B. The yellow bases of Ochrosia elliptica were re-investigated with the result that formula revisions were indicated. Ellipticine has been shown to be $C_{17}H_{14}N_2$ and methoxyellipticine is $C_{18}H_{16}ON_2$. Ellipticine has been compared directly with the alkaloid QBIII isolated from a species of Aspidosperma by Dr. F. A. Hochstein of Pfizer. (Hochstein has also isolated a colorless alkaloid QBI, $C_{18}H_{20}N_2$, which on catalytic dehydrogenation is converted into QBIII. QBI has a typical carbazol spectrum and, therefore, ellipticine is considered to be a pyridocarbazol Hochstein has collected samples of practically all of the possible linear and angular pyridocarbazols for ultraviolet spectral comparisons and, as a result, many possible structures for ellipticine have been eliminated and one in particular is strongly suggested).

C. Lunasia amara.

(1) Isolation studies were continued and resulted in a total of eleven alkaloids of which five have been mentioned in a previous report. Of the new ones, two are furoquinolines of known structures -- skimmianine and kokusagine; two-lunine and lunamarine - have been isolated by earlier workers and are now characterized in greater detail; the remaining two - LA-3 and LA-10 -- are only partially characterized and will not be discussed further at this time. Lunine which was described previously only by melting point has been shown to have the following features; $C_{16}H_{17}O_4N$, no methoxyl group, one N-methyl group, at least 1C-methyl group, optically active ($[\alpha]_D$ - 39°), probably a 4-quinolone according to the ultraviolet and infrared spectra. This alkaloid may well be the methylenedioxy analog of lunacrine. Lunamarine, previously described by empirical formula, m.p. and rotation (inactive) has now been shown to contain one methoxyl and one N-methyl group. The infrared spectrum is very similar to that of iso-LA 4 including the methylenedioxy group. The ultraviolet spectrum supports the 4-quinolone system, although the UV in acid solution is shifted to longer wavelengths by about 20 mμ. This phenomenon is adequately explained by the additional

oxygen substituents. On the basis of ultraviolet studies it is proposed that lunamarine is 7-methoxy-1-methyl-2-(3',4'-methylenedioxyphenyl)-4-quinolone.

(2) Extensive studies have been made toward elucidating the structure of lunacrine. The original proposal of a dihydrofuroquinolone ring system has been substantially confirmed by the following series of reactions. Lunacrine on refluxing with 15% potassium hydroxide is converted to an alkali-soluble substance, $C_{16}H_{21}O_4N$, which on treatment with diazomethane afforded an oily product which formed a crystalline perchlorate m.p. 150°. The free base obtained from the perchlorate in quantitative yield crystallized easily, m.p. 85°, $C_{17}H_{23}O_4N$, and had an ultraviolet spectrum characteristic of a carbostyril. These reaction products are explained in terms of the following transformation: a dihydrofuroquinolone ---> a 4-hydroxycarbostyril ---> a 4-methoxy carbostyril. The compound, m.p. 85°, on treatment with α-toluenesulfonyl chloride yielded a base, m.p. 118°, identical in IR, UV, and paper chromatography with lunacrine but having the opposite sign of rotation. Crystallization of an equal weight mixture of the product and natural lunacrine gave a crystalline substance melting at 148° and optically inactive showing that the molecule has only one assymmetric center and thus the reaction product is the enantiomorph of natural lunacrine. This product has also been obtained by fusion of the perchlorate, m.p. 150°, described above, followed by treatment of the crystalline product, m.p. 196°, with lithium bromide in acetonitrile under reflux. The rationale being that the fusion product, m.p. 196° is a 4-methoxydihydrofuroquinolinium perchlorate and that the bromide ion attacks the methyl of the 4-methoxy group forming methyl bromide and the N-methyl-dihydrofuro-4-quinolone, in other words, an E_2 elimination of a methyl group or an S_N2 displacement of quinolinolate. The inversion which occurred in this sequence of reactions is assumed to have taken place in the ring closure reaction.

The carbostyril, m.p. 85° was also formed by treatment of the methiodide of lunacrine with dilute alkali, a reaction series suggested by the work of J. R. Price in Australia who is also working with these alkaloids. It has further been shown that the compound m.p. 85° is identical with the alkaloid lunacridine described by earlier workers. (The Australian workers have shown that lunacridine is in all probability an artefact of the isolation manipulations and not a natural product.)

Direction of Current Research:

A. Ocotea leucoxylon. Further studies toward complete structure determination will depend upon the outcome of the pharmacological studies.

B. The yellow bases of Ochrosia elliptica. Nuclear magnetic resonance spectral studies will be carried out in order to established whether ellipticine contains one ethyl substituent or two methyl substituents. (F.A. Hochstein is working on the synthesis of the parent pyridocarbozol suggested by his UV studies).

C. Lunasia amara.

(1) The isolation has been completed. The alkaloids La-3 and LA-10 will be further characterized and their structures studied (more plant material will have to be secured and the alkaloids isolated). The structure of lunine will be studied when more material is available. The proposed structure of lunamarine will be synthesized.

(2) Further degradative reactions will be carried out with lunacrine in order to establish the position of the methoxyl group and the nature of the side chain on the dihydrofuro moeity (isopropyl has been proposed). Model compounds will be synthesized for ultraviolet spectral comparisons. Attempts will be made to determine the mechanism leading to epimerization in the sequence of reactions discussed above for lunacrine.

Part B included: Yes [XX] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Goodwin, Sidney, Smith, A. F., and Horning, E. C., Alkaloids of Lunasia Amara. 4-Methyl-2-phenylquinoline. J. Am. Chem. Soc. 79, 2239 (1957).

Goodwin, Sidney and Witkop, Bernhard, Quinol Intermediates in the Oxidation of Phenols and their Rearrangements. ibid., 79, 179 (1957).

Denss, R, Häfliger, F. and Goodwin, Sidney, Über Derivate des Phenylbutazons. II. Derivate mit einer Hydroxylgruppe in des 4-standigen Seitenkette. Helv. Chim. Acta, 40, 49 (1957).

Form No. ORP-2
Oct. 1957

Serial No. NHI-28
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: A. Study of charge-transfer complexes.
B. Fundamental Studies on Surface Phenomenon.

Principal Investigators: Warner L. Peticolas, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years (Calendar year 1957):
Total: 1
Professional: 1
Other:

Patient Days (Calendar year 1957):
None

Project Description :

A. Studies of complex formation were completed. A number of experiments on chloranil complexes were run, but no new information resulted. The electron localization hypothesis is in accord with current information on complex formation. The correlations obtained during this work have been published.

B. Experimental studies of the absorption of amino acids and simple peptides on nucleic acids were continued. In the course of this work it became apparent that the theoretical aspects of adsorption required further study. In particular, it was necessary to consider the effect of perturbations of the adsorbent by the adsorbate. An extension of the Brunauer-Emmett-Teller equation was therefore proposed.

Direction of Current Research: Projects terminated.

Part B included: Yes [XX] No [ ]

Serial No. NHI-28

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Peticolas, W. L., Charge-Transfer Complexes between Chloranil and Polycyclic Aromatic Hydrocarbons, J. Chem. Phys., 26, 429 (1957).

Peticolas, W. L., Effect of Adsorbent Perturbation on B.E.T. Theory, J. Chem. Phys., 27, 436 (1957).

Serial No NHI-29
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Structural Studies on Andromedotoxin (The project was started in July, 1957).

Principal Investigator: Charles C. Sweeley, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years (Calendar year 1957):
Total: 0.3
Professional: 0.3
Other:

Patient Days (Calendar year 1957):
None

Project Description:

Andromedotoxin ($C_{22}H_{36}O_7$), isolated from Rhododendron, had previously been studied by V. Stromberg and W. H. Tallent. The polycyclic compound was found to contain five or six hydroxyl groups, one of which occurred as an acetate ester in the natural product. Two hydroxyl groups were found to form a 1,2 glycol which could be cleaved by periodate. Recent studies on andromedotoxin and the des-acetyl derivative, andromedol, have resulted in isolation of pure, crystalline periodate oxidation products with formulas $C_{22}H_{34}O_7$ (I) and $C_{20}H_{32}O_6$ (II) respectively. Infrared spectra indicated that the products (I and II) contained one carbonyl group as a result of periodate cleavage.

A second method of oxidation utilized chromic acid in pyridine to effect a multiple oxidation of andromedol and andromedotoxin. The products (III, IV) were isolated and infrared spectra indicated two or more carbonyl groups as a result of oxidation.

Chromic acid in pyridine was used to further oxidize the periodate oxidation product of andromedol. (II) The product of this reaction (V) was isolated in impure form and infrared and ultraviolet spectra indicated the presence of a cyclopentadiene ring in addition to several carbonyl groups.

Direction of Current Research: It is hoped that V will be isolated in pure form on a scale large enough so that this compound may be further characterized by physical and chemical means. Attempts will be made to

aromatize various of these oxidation products. The aromatized compounds will be used to establish the nature of the polycyclic ring system of andromedotoxin. The assignment of hydroxyl group positions will be left for further work on the intact structure.

Part B included: Yes ☐ No ☒

Form No. ORP-2
Oct. 1957

Serial No. NHI-30
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the Metabolism of m-tyrosine in vivo.
(These investigations were started in January, 1957).

Principal Investigator: C. C. Sweeley, Ph.D.

Other Investigators: M. S. Bronk

Cooperating Units: Facilities of Dr. M. Cotten, Section on Physiology, Laboratory of Chemical Pharmacology, utilized for the animal work.

Man Years (Calendar year 1957):
Total: 0.4
Professional: 0.1
Other: 0.3

Patient Days (Calendar year 1957):
None

Project Description:

Studies by M. Cotten on the intravenous injection of m-tyrosine into dogs indicated a strong central nervous system action of m-tyrosine or a metabolite at a relatively low level (effects were noted with 10.0 mg/kg of dl amino acid injected in 2-5 minutes). It was of interest to study the nature of the metabolic end-products in urine after m-tyrosine injection.

Major Findings: Acids and bases were extracted from dog urine collected for four hours after m-tyrosine injection. Amino acids were removed from the urine by adsorption onto deactivated charcoal.

Paper chromatographic examination of the urine extracts indicated that m-tyrosine was metabolized mainly to m-tyramine and m-hydroxy phenylacetic acid. However, small quantities of an unknown base and an unknown amino acid were found in the extracts.

When the amino acid was purified by chromatographic techniques, paper chromatography comparisons indicated that the metabolite was not synthetic N-methyl-m-tyrosine or synthetic N-acetyl-m-tyrosine.

Direction of Current Researches: Attempts to identify the unknown metabolites of m-tyrosine are currently in progress.

Part B included: Yes ☐ No ☒

Form No. ORP-2
Oct. 1957

Serial No. JJT-31
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the Human Excretion of m-hydroxyphenyl-hydracrylic Acid. (These studies were initiated in November, 1956).

Principal Investigator: C. C. Sweeley, Ph.D.

Other Investigators: M. S. Bronk

Cooperating Units: None

Man Years (Calendar year 1957):
Total: 0.6
Professional: 0.2
Other: 0.4

Patient Days (Calendar year 1957):
30

Project Description:

In the course of preliminary studies on the occurrence of various phenolic acids in human urine, reports from other laboratories were confirmed; certain phenolic acids related to m-tyrosine, or some other m-hydroxyphenyl metabolite, seemed to be present in greater than normal concentrations. A two dimensional paper chromatography system was used to separate the various acid metabolites from phenylalanine, tyrosine and tryptophan and individual spots were assayed with a spectrophotofluorometer. The daily urinary levels of a few of the metabolites were obtained in this manner.

Major Findings: After a preliminary screening program of about twenty schizophrenic patients at St. Elizabeth's Hospital, two male patients were placed on hospital diets at NHI, along with two normal male subjects. The diets consisted of known quantities of normal foods and the quantity and composition of meals were held constant throughout the study. Table I is a summary of the excretion data for m-hydroxyphenylhydracrylic acid (m-HPA) (which at the time was an unknown m-hydroxyphenyl metabolite).

Table I

Subject	Days on Diet	Excretion (mg/gm creatinine) Average	High	Low
Control A	7	9.5	13.2	6.6
Control B	20	10.2	24.7	5.7
Patient A	20	35.3	64.9	12.2
Patient B	13	53.0	89.2	38.0
Patient B (no diet control)	16	62.5	126.1	27.4

In addition, the variation in excretion of m-HPA was followed during the course of the day; these studies indicated an excretion pattern unrelated to diet for normals and patients, i.e., there was no correlation with creatinine excretion and the substance was excreted in greater quantities from 8 P.M. to midnight than at other times of the day.

Degradation studies and synthetic studies (the latter work was done by Mrs. R. J. Highet) established the structure of the metabolite as m-HPA. This work was completed at a time when M. Armstrong reported on the isolation and structure determination of the same metabolite from human urine. Armstrong was of the opinion that m-HPA excretion reflected the dietary intake of a simple aromatic acid and was in no way connected with m-tyrosine metabolism in humans.

Experiments were conducted in this laboratory using Armstrong's synthetic diet (lactalbumin, minerals, vitamins, sucrose and Wesson oil) and his studies were confirmed; normal controls and schizophrenic patients on the synthetic diet excreted only very small quantities of m-HPS (of the order of 2-3 mg/day) after two days on the diet.

The differences in daily excretion levels of m-HPA in normals and patients used in the study were real and considerable. Interpretation of the data is made difficult in view of the later experiments with synthetic diets. The question of the dietary precursor is of some interest but remains unanswered. No conclusions may be drawn at this time concerning the possible implication of m-tyrosine in normal human metabolism

Part B included: Yes ☐ No ☒

Form No 088-2
Oct. 1957

Serial No. NHI-32
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Chemical and Enzymatic Transformations of Amine Oxides. (This work was started in August, 1955.)

Principal Investigator: C. C. Sweeley, Ph.D.

Other Investigators: R. Levenberg

Cooperating Units: None

Man Years (Calendar year 1957):
Total: 0.43
Professional: 0.2
Other: 0.23

Patient Days (Calendar year 1957):
None

Project Description:

Amine oxides are prepared chemically by hydrogen peroxide oxidation. The chemical rearrangement reactions are studied in a micro-chemical system employing ferric ion complexed with tartaric acid. The reaction mixtures are examined by paper chromatographic techniques.

Major Findings: Nicotine oxide has been prepared and the chemical rearrangement reaction was studied. Several products resulted from a ferric ion catalyzed reaction.

Morphine oxide was prepared and studied in the same manner. This oxide was found to resist rearrangement under mild conditions.

Direction of Current Research: The nature of the reaction products resulting from the chemical rearrangement of nicotine oxide will be studied.

A strain of Pseudomonas was found by Japanese workers to degrade nicotine by oxidative cleavage of the pyrrolidine ring. The implication of nicotine oxide as a biological intermediate in this reaction was suggested.

Studies have been initiated in this laboratory to look for more degradation products by the Pseudomonas system and to isolate the enzyme systems involved in the oxidative cleavage of the pyrrolidine ring. It is hoped that this (adaptive) enzyme system will provide a unique tool for studying the enzymatic mechanism of N-oxide rearrangement.

Serial No. NHI-32

Part B included: Yes /XX/ No / /

Serial No. NHI-32

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Sweeley, C. C. and Horning, E. C. Rearrangement and Decarboxylation Reactions of N,N-Dimethylglycine Oxide, J.A.C.S., 79, 2620 (1957).

Form No. ORP-2
Oct. 1957

Serial No. NHI-33
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Investigation of Immobile Phases Suitable for the Gas-liquid Partition Chromatography of Fatty Acid Esters. (This work was started in September, 1957).

Principal Investigator: C. C. Sweeley, Ph.D.

Other Investigators: R. Levenberg

Cooperating Units: Dr. S. R. Lipsky, Yale University Medical School, New Haven, Conn.

Man Years (Calendar year 1957):
Total: 0.2
Professional: 0.1
Other: 0.1

Patient Days (Calendar year 1957):
None

Project Description:

A need for better partition agents for the complete gas chromatographic separation of methyl esters of fatty acids has stimulated an investigation in this laboratory of the properties of various steroids in this connection. Two model compounds, cholestanone and cholestanyl methyl ether have been synthesized from dihydrocholesterol. These materials were sent to Dr. S. R. Lipsky at Yale University Medical School for testing with Burrell gas chromatography equipment. Preliminary results indicated that cholestanyl methyl ether supported on celite will provide better separation of the C^{18} components (oleic, linoleic and stearic acids) of a mixture than Apiezon L grease on celite, currently the most widely used partition agent for fatty acid analysis.

Direction of Current Researches: Further studies, in collaboration with Dr. Lipsky, with cholestanyl methyl ether will be necessary to establish the optimum conditions for operation of the gas chromatography. The effects of altering stereochemical configuration and varying the number and type of functional groups in the steroid molecule will be studied in order to determine the structural features necessary for best separations.

Part B included: Yes [ ] No [XX]

Form No. ORP-2
Oct. 1957

Serial No. NHI-34
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Enzymatic Studies on the N-methylation of Amines and Amino Acids. (These studies were started in September, 1957).

Principal Investigators: C. C. Sweeley, Ph.D.
M. S. Fish

Other Investigators: M. S. Bronk
E. P. Lawrence

Cooperating Units: None

Man Years (Calendar year 1957):
Total: 0.4
Professional: 0.1
Other: 0.3

Patient Days (Calendar year 1957):
None

Project Description:

Studies on the mechanism of exidative N-demethylation of amines and methylamino acids by chemical and enzymatic processes have been carried out in this laboratory for the past two years. Studies recently initiated have been designed to determine the mechanism of N-methylation. In vitro experiments using mouse liver homogenates with the appropriate cofactors and various substrates were carried out and the metabolites were examined by paper chromatographic techniques.

Major Findings: In the presence of active methionine, produced from ATP, methionine and magnesium ion, mouse liver homogenates catalyzed the metabolism of m-tyrosine and p-tyramine to unknowns. Other substrates, tyrosine, N-methyltyrosine and histidine, showed no evidence of methylation.

Direction of Current Research: Studies directed to the identification of the metabolites of m-tyrosine and tyramine are in progess. Attempts will be made to improve the enzymatic yield by the choice of optimum conditions and the enzyme systems will be located by fractionation studies on whole homogenate.

Part B included: Yes ☐ No ☒ XX

Form No. ORP-2
Oct. 1957

Serial No. NHI-35
1. Laboratory of Natural Products
2. Natural Products
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on Alkaloids of the Amaryllidaceae; to examine the alkaloids of Crinum Moorei (Hook) f.

Principal Investigator: J. R. Crowder, Ph.D.

Other Investigators: Isolations were carried out by Dave Rogerson and staff.

Cooperating Units: None

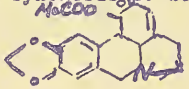
Man years (Calendar year 1957):
Total: 1
Professional: 1
Other:

Patient Days (Calendar year 1957):
None

Project Description:

Alkaloidal extracts from the seeds of Crinum Moorei (Hook) f. were separated into their components. Lycorine, powelline, crinine and crinamidine were all found and in addition, three new alkaloids were isolated.

One of these was completely investigated and was shown by degradation and partial synthesis to be 1-acetyl lycorine (I)



I

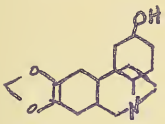
The second was shown to possess properties like those of crinamidine. It has the formula $C_{17}H_{19}NO_5$, rotation $[\alpha]_D = +35.2^0$, and had one methoxy-, one methylenedioxy- and one oxidizable hydroxy-group. Insufficient material was available for a thorough investigation.

The third was imperfectly characterized, since it was obtained in minute quantity and may be an impure polymorph of the second. It possessed one methoxy-group and had a positive rotation.

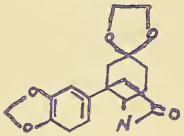
Alkaloidal extracts from the bulbs of Crinum Moorei (Hook) f. yielded another new alkaloid, shown to be phenolic, and probably having a lycorine-type ring system. Work is still in progress on this alkaloid.

Synthetic attempts to prepare oxodihydrocrinine to confirm the

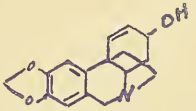
structure of crinine as (II) had proceeded to the saturated lactam (III) but were abandoned when work in this laboratory indicated that the oxygen function in crinine is as shown in (IV).



II



III



IV

Investigations of the structure of crinamidine have yielded several new degradation products of doubtful character and no further evidence has been found to advance knowledge of its structure.

Direction of Current Research: To complete investigation of the new alkaloid from Crinum Moorei bulbs, and to continue the investigations on crinamidine.

Part B included Yes ☐ No ☒

Form No. ORP-2
Oct. 1957

Serial No. NHI-36
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the Structure of the Alkaloids derived from *Cassia excelsis*.

Principal Investigator: E. J. Highet, Ph.D.

Other Investigators: P. F. Highet, A.B.

Cooperating Units: None

Man Years (Calendar year 1957):
Total: 1.50
Professional: .75
Other: .75

Patient Days (Calendar year 1957):
None

Project Description:

1. A method has been perfected for the preparation of two pure materials comprising a major portion of the alkaloidal extract of *Cassia excelsis*. The dried leaves of the plant are ground and extracted by hot ethanolic tartaric acid, and the basic constituents isolated by conventional methods in 2.2% yield. This extract is triturated with cyclohexane and the soluble material converted to the hydrochloride and crystallized from ethanol. Silicic-acid chromatography of the crystalline hydrochloride, or, preferably, of the regenerated bases, yields casselsine and cassine in 0.17% and 0.28% yields, respectively; the mother liquors of the hydrochloride crystallization contain very little cassine and casselsine, and have not been extensively investigated.

2. Characterization of cassine and casselsine show them to be representatives of a new type of alkaloid; tentative formulae have been assigned to them as homologues, with the composition $C_{19}H_{37}NO_2$ and $C_{20}H_{39}NO_2$, respectively. The materials have very similar chemical properties: Formation of neutral diacetates shows each to possess a secondary amino function and a primary or secondary hydroxyl. Positive iodoform reactions, the preparation of semicarbazones and bis-piperonylidine derivatives show each to possess the group $-CH_2COCH_3$. The failure of the bases to absorb hydrogen over palladium catalyst show the absence of double bonds; this fact and the elemental composition imply the presence of a carbocyclic ring in each.

The physical properties of these alkaloids and their derivatives

are here tabulated. The infrared absorption of the Nujol mulls of the hydrochlorides about 10 μ affords the most satisfactory way of distinguishing the two materials:

	Cassine	Casselsine
M. P.	55-58°	66-67°
Rotation		
Hydrochloride, m.p.	172-173.5°	172-173°
Semicarbazone, m.p.	139-141°	141.5-142.5°
Diacetate semicarbazone, m.p.	115-117°	123-124
bis-piperonylidine derivative, m.p.	106-107°	96-97°
IR, ca. 10 μ	0.90, 10.05 μ	9.88, 10.02, (weak) 10.10

Cassine is dehydrogenated by 10% palladized charcoal under nitrogen at 230°. The product, dehydrocassine, is evidently the result of rearrangement and degradation, for it corresponds to $C_{18}H_{29}NO_2$ and has lost its N-CH_3 group. That it retains the ketonic group is shown by the carbonyl absorption in the infrared. Its ultraviolet spectrum closely resembles that of 3-hydroxypyridine, and dehydrocassine shares with 3-hydroxy pyridine the unusual property of showing bathochromic shifts in both acid and basic solutions. The implication that this materials is a substituted 3-hydroxypyridine is corroborated by the formation of a basic acetate with a new carbonyl absorption at 5.70 μ.

Casselsine forms a dehydrogenation product with similar properties, which has been shown to be different from dehydrocassine, but has not yet been more fully investigated.

Direction of Current Research: Terminated

Part B included: Yes ☐ No ☒

Form No ORP-2
Oct 1957

Serial No NHI-37
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: A. New Methods of Synthesis of Polycyclic Compounds

B. Novel Hydrogenation Methods

Principal Investigators: Gordon N. Walker, Ph.D.

Other Investigators: None

Cooperating Units: None

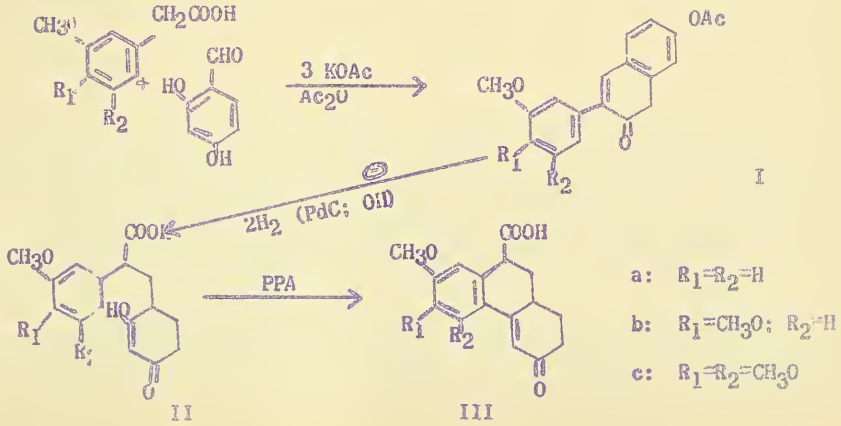
Man Years (Calendar year 1957):
Total: 0.5
Professional: 0.5
Other:

Patient Days (Calendar year 1957):
None

Project Description:

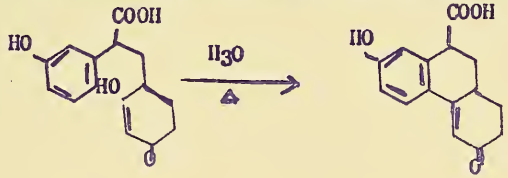
General Purpose of Research: To develop new synthetic means for preparation of chemically and medicinally important tricyclic structures emphasis being laid upon improved hydrogenation methods in getting key intermediates.

Progress during Past Six Months: A new sequence of reactions, leading in three steps to some important methoxyhydrophenanthrene ketones, has been discovered:



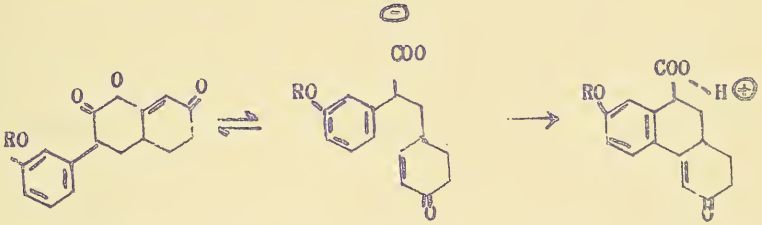
The first step in this sequence is a new Perkin condensation of 2,4-dihydroxybenzaldehydes with phenylacetic acids. It was found that good yields of 3-aryl-7-acetoxycoumarins (I) were obtained when at least three moles of potassium acetate were used in carrying out this condensation. The second step is a novel hydrolytic hydrogenation in which the cinnamic double bond and the resorcinol moiety are reduced simultaneously in the presence of palladium-charcoal and dilute sodium hydroxide solution, giving substituted dihydroresorcinols (II). This unusual reaction has some features in common with the modern preparation of dihydroresorcinol itself. The third step, cyclodehydration of II, was effected with polyphosphoric acid, and gave the desired hydrophenanthrene keto-acids, III.

Proof for the structures of compounds III was obtained by decarboxylation to corresponding ketones, and by aromatization and decarboxylation to corresponding methoxy-phenanthrols. It was also found that the sequence is applicable to hydroxy-substituted A-ring compounds, viz:



IV V

At present it seems likely that these cyclizations depend upon a hitherto unknown mechanism involving intermediate enol-lactone and vinylogous carbonylium ion formation:



One reason for believing that such a mechanism operates here is the important fact that the present cyclodehydration, unlike two other cyclizations described previously involving attack of cyclic 1,3-diketones upon an aromatic ring, is applicable to synthesis of the sterically relatively inaccessible compound IIIc, as well as to IIIa and IIIb. The classical cyclodehydration steps involving intermediate hydroxyketone are very unlikely to be implicated IIc IIIc and IV V.

Direction of Future Research: While specific ideas cannot be discussed here, one can be sure that the concepts involved in the work described briefly above will be helpful in future studies in polycyclic compound chemistry. Now that examples illustrating the feasibility of

all three categorical 1,3-diketone cyclodehydrations have been provided, the work of the future in this area will perhaps consist of elaboration, rather than further discovery. Cyclizations of other compounds involving presumed intermediary enol-lactones are conceivable, and certainly further applications of the improved Perkin condensation, the novel reduction of 1,3-diphenols to 1,3-diketones, and the boron trifluoride-catalyzed cyclodehydrations described in earlier reports should be envisioned and developed. In a broad sense, compounds III and those obtained from similar cyclizations are related in several respects both to known mitosis poisons and to the ubiquitous steroid nucleus, and thus there is some assurance that compounds gotten through these syntheses will in time be found to have interesting and possibly unusual physiological activity.

Part B included: Yes [XX] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Walker, G. N., Synthesis of 1,2,3,9,10,10a-Hexahydro-3-Ketophenanthrenes from 3-Aryl-7-Acetoxycoumarins, J. Am. Chem. Soc. 79, 1772 (1957).

Walker, G. N., Synthesis of Methoxyhydrophenanthrenes and Methoxyhydrocyclohepta(a)naphthalenes by Acylation of Ketones with Homoveratric Anhydride, J. Am. Chem. Soc. 79, 0000 (1957).

Form No. ORP-2
Oct. 1957

Serial No. NHI-38
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Isolation of Andromedotoxin from Rhododendron and Related Genera.

Principal Investigator: David L. Rogerson, Jr., B.S.

Other Investigators: James D. Link, B.S.
Douglas L. Johnson

Cooperating Units: Dr. John C. Keresztesy, NIAMD, Henry E. Lutterlough and James M. Miles, Pilot plant equipment and operation.

Man Years (Calendar year 1957):
Total: .15
Professional: .05
Other: .10

Patient Days (Calendar year 1957):
None

Project Description:

Temporarily the isolation of Andromedotoxin has been discontinued and the materials received to date for processing in bulk are as follows: 7422 lbs. Rhododendron maximum, 705 lbs. Kalmia latifolia and 350 lbs. Kalmia angustifolia, var. caroliniana. In addition, 20 more members of the Ericaceae family have been investigated for andromedotoxin content on a one pound level or less.

Direction of Current Research: The isolation of andromedotoxin will be resumed upon receipt of plant material now on order.

Part B included: Yes [ ] No [XX]

Form No. CRP-2
Oct. 1957

Serial No. NHI-39
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Isolation of Constituents from Human Urine and Animal Tissue.

Principal Investigator: David L. Rogerson, Jr., B.S.

Other Investigators: James D. Link
Douglas L. Johnson

Cooperating Units: None

Man Years (Calendar year 1957):
Total: .45
Professional: .20
Other: .25

Patient Days (Calendar year 1957):
None

Project Description:

Approximately 200 l of human urine have been partially or completely processed for constituents, mainly organic acids and bases. Also, an additional 300 l of urine obtained exclusively from males, have been collected locally for processing.

To date, over 1200 lbs. of fresh, frozen veal brains and 20 lbs. of frozen pork chitterlings have been processed for indolic compounds, which is only a slight increase over the previous report as recent isolations have been of an exploratory nature on a relatively small scale.

Direction of Current Research: Upon completion of current researches for improved isolation techniques, large scale processing of fresh, frozen veal brains will be resumed.

Part B included: Yes ☐ No ☒

Form No. ORP-2
Oct. 1957

Serial No. NHI-40
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Isolation of Alkaloids, Glycosides and Other Constituents from Plant Material.

Principal Investigators: David L. Rogerson, Jr., B.S.

Other Investigators: James D. Link
Douglas L. Johnson
John C. Keresztesy, Jr. (summer employee)

Cooperating Units: Dr. John C. Keresztesy, NIAMD, and Staff. (Henry E. Lutterlough, James M. Miles) Large scale equipment.
Dr. B. G. Schubert, U. S. Department of Agriculture, Plant Accessions and Idantifications.

Man Years (Calendar year 1957):
Total: 1.05
Professional .50
Other: .55

Patient Days (Calendar year 1957):
None

Project Description:

The extraction of alkaloids from plant materials is generally conducted employing either ethanol or a 1% ethanolic tartaric acid solutions with an occasional small scale extraction using a 65% chloroform-tetrahydrofuran mixture. Also, trichloroethylene, chloroform and chloroform-ethanol mixtures are used at selected steps in the general procedure. Recently 2N sulfuric acid has been substituted for 2N hydrochloric acid and concentrated ammonium hydroxide for solid sodium carbonate at specific points.

To date, 16 materials have been completely or partially processed for glycosides and 208 for alkaloids, which is an increase of 27 since December 31, 1956. Included in the 208 isolations for alkaloid are 17 materials each from the Puerto Rican and Costa Rican investigations and 12 from the Mexican project. Also, 14 supplementary shipments totaling 512 lbs. have been processed for materials currently under investigation.

Complete or partial processing for desired constituents other than alkaloids and glycosides has been conducted on 7 plant samples.

Direction of Current Research: Stress will be placed upon Mexican plant materials which have had favorable initial observations.

Efforts will be made to improve isolations by modifying procedures and designing new equipment, particularly with regards to liquid-liquid extraction.

Part B included: Yes ☐ No ☒

Form No. ORP-2
Oct. 1957

Serial No. NHI-41
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Testing of Fresh Plant Material and Herbarium Samples for Alkaloids and Glycosides.

Principal Investigators: David L. Rogerson, Jr., B.S.

Other Investigators: James D. Link
Douglas L. Johnson
John C. Keresztesy, Jr. (summer employee)

Cooperating Units: Plant accessions and identifications were by Dr. B. G. Schubert, Plant Industry Station, U. S. Department of Agriculture, Beltsville, Maryland.

Man Years (Calendar year 1957):
Total: .25
Professional: .25
Other:

Patient Days (Calendar year (1957):
None

Project Description:

To date, 2571 plants have been tested for alkaloids which is an increase of 497 since December 31, 1956. This increase includes 91 plants of African origin received from the School of Tropical and Preventive Medicine, Loma Linda, California and 366 plants screened in Costa Rica and Mexico by this investigator. The positive analyses numbered 77, which is 15.5% of the number tested. In addition, 30 supplementary shipments of previously tested plant materials were received which raises this total to 277.

The screening of plants for glycosides and the determination of alkaloids in Herbarium samples has been temporarily discontinued and the results remain as previously reported, which are as follows: 36 (68%) of the 53 materials selected for glycoride testing were found to contain active glycosides (Glycoride tests on 5 new samples are in progress) and of the 524 Herbarium samples screened, 129 (24.6%) were found to contain one or more alkaloids.

Direction of Current Researches: Tentative plans call for this investigator to return to Mexico and screen additional plant materials for alkaloids.

Part B included: Yes ☐ No ☒

Form No. ORP-2
Oct. 1957

Serial No. NHI-42
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Synthesis of Organic Compounds and the Preparation of Adsorbents.

Principal Investigator: David L. Rogerson, Jr., B.S.

Other Investigators: James D. Link

Cooperating Units: None

Man Years (Calendar year 1957):
Total: .10
Professional: .05
Other: .05

Patient Days (Calendar year 1957):
None

Project Description:

The synthesis of organic compounds for starting materials and reference compounds has been conducted. Specifically, gramine and diethyl dimethylaminomalonate have been prepared in quantity.

Cotton acid succinate and various forms of deactivated charcoal have been prepared for use as adsorbents in chromatographic columns. The deactivated charcoal preparations include 4,6 and 10% stearic acid and 5% octadecylamine.

Direction of Current Research: Organic compounds and substances will be synthesized and prepared in all desired quantities upon request.

Part B included: Yes ☐ No ☒

Form No. ORP-2
Oct. 1957

Serial No. NHI-43
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Isolation of Organic Bases (other than Indoles) from Human Urine.

Principal Investigator: M. S. Fish, Ph.D.

Other Investigators: N. Marie Johnson

Cooperating Units: None

Man Years (Calendar year 1957):
Total: .25
Professional: .15
Other: .10

Patient Days (Calendar year 1957):
None

Project Description:

The isolation procedure described above was designed as a general one. Experimental conditions such as pH and partition coefficients were adjusted to such values as would insure extraction of any organic-soluble base present. While the chromatographic conditions were set up for the purpose of obtaining a high recovery of indole bases, it was found that other non-indole bases were present in other fractions of the eluate. The exact nature of these materials is not yet known, although paper chromatographic systems have been worked out for their separation.

Direction of Current Researches: The characterization and attempted identification of these materials is underway.

Part B included: Yes ☐ No ☒

Form No. ORP-2
Oct. 1957

Serial No. NHI-44
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Synthetic Work Related to the Identification of Metabolites.

Principal Investigator: M. S. Fish, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years (Calendar year 1957):
Total: .20
Professional: .20
Other:

Patient Days (Calendar year 1957):
None

Project Description:

This project is of the type that operates according to the needs of this and other laboratories. The preparation of reference compounds for comparison with those isolated from natural sources and of materials for physiological study is frequently undertaken. For example, 3-methoxy-4-hydroxymandelic acid was recently prepared for use by Dr. Sjoerdsma of the Heart Institute and for investigators in other Institutes.

Direction of Current Research: This project will continue as the need arises. The presence of methylated histamine compounds in urine has recently been reported. As none of the reference compounds are available at present, it is likely that some of these materials will be prepared in this laboratory. Other syntheses will be undertaken for the purpose of identifying the unknown bases described previously.

Part B included: Yes ☐ No ☒

Form No. ORP-2
Oct. 1957

Serial No. NHI-45
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A

Project Title: Enzymatic N-methylation-demethylation Studies.

Principal Investigator: M. S. Fish, Ph.D.

Other Investigators: Esther P. Lawrence, B.S.

Cooperating Units: This project is in collaboration with Dr. C.C.Sweeley

Man Years (Calendar year 1957):
Total: 0.15
Professional: .5
Other: .10

Patient Days (Calendar year 1957):
None

Project Description:

Since the isolation of N-methylated bases from plant sources in this laboratory some time ago, chemical and enzymatic N-methylation and demethylation reactions have been of primary interest. Enzymatic N-demethylation of dimethylamino acid oxides was reported recently from this laboratory. Mouse liver homogenate with appropriate cofactors was utilized for this purpose. Studies are now underway to determine the enzymatic requirements for N-methylation of bases and amino acids. The need for such studies is obvious now that the methylated histamines and tryptamines (as well as epinephrine) are known to be present in humans.

Direction of Current Research: It is currently being determined if certain observations made recently in this laboratory indicate that enzymatic N-methylation of various indole bases has been successful. If so, the optimum conditions for such reactions will be determined.

Part B included: Yes ☐ No ☒

Form No. ORP-2
Oct. 1957

Serial No. NHI-46
1. Laboratory of Natural Products
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Procedures for the Isolation of Organic Bases from Natural Sources.

Principal Investigator: M. S. Fish, Ph.D.

Other Investigators: N. Marie Johnson, B.A.
Esther P. Lawrence, B.S.

Cooperating Units: None

Man Years (Calendar year 1957):
Total: 2.40
Professional: 60
Other: 1.80

Patient Days (Calendar year 1957):
None

Project Description:

A study of the different methods currently in use for the isolation of bases from natural sources has revealed two principal shortcomings: The procedures which were efficient (capable of isolating a material present in very small concentration) were usually specific for a single known material or a limited number of related materials. The more generalized procedures usually lacked the efficiency of the specialized ones and the "trace" materials were extracted in poor yield or not at all. The present project was intended to overcome these difficulties as far as possible and from that point of view, has been successful. During the past year a whole spectrum of hitherto unknown bases has been isolated from human urine and it is hoped that the method can be extended to other areas as well. In general, the procedure involves a preliminary extraction, followed by purification over a column, affording material which can be assayed quantitatively by use of the spectrophotofluorimeter. Re-extraction and paper chromatography are then utilized for qualitative purposes.

By use of the procedure described above, it has been possible to isolate and identify, from human urine, the following bases:

Serotonin (already known to be present)
Tryptamine (identification previously in question)
N,N-dimethyltryptamine oxide (The identification work on
Bufotenine these not yet completed, but fairly certain)

In addition, there is an unknown base, apparently, closely related to serotonin, which is being characterized. Also, the oxide of bufotenine appears to be present as well as two or three other materials of which little is known as yet.

Direction of Current Research: It is intended to complete the the identification of the two dimethylated bases and to characterize and identify, if possible, the other materials.

Part B included Yes ☐ No ☒

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 1958

Total:	**$233,635**
Direct:	**$168,167**
Reimbursements:	**65,468**

Form No. ORP-2
Oct. 1957

Serial No. NHI-47
1. Laboratory of Cellular Physiology and Metabolism
2. Cellular Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies of the Mode of Involvement of Genetic Material in Synthesis.

Principal Investigators: William J. Dreyer, Christian B. Anfinsen, and Arnold M. Katz (July to December)

Other Investigators: Judith Wegman (Technical - summer)

Cooperating Units: None.

Man Years (Calendar year 1957):
Total: 2.15
Professional: 1.9
Other: .25

Patient Days (Calendar year 1957):
None.

Project Description: The effect of genetic mutations in the T_2 bacterial virus on the structure of virus protein.

Progress during past year: 1. Procedures have been developed which enable us to isolate 10-20 gram quantities of highly purified varus. Purified preparations of several mutant forms of the T_2 virus have been made.

2. Methods have been developed which allow us to separate the virus proteins from nucleic acids.

3. Reagents have been found which enable us to deaggregate the proteins of the virus coat without losing the antigenic reactivity of these proteins.

4. Physical and chemical studies of the proteins have been started. Terminal group and amino acid analyses have been carried out. Ultracentrifugal, electrophoretic, and osmotic pressure measurements have been performed. These studies, although not yet complete, indicate that there are very few species of protein molecules in the virus.

5. Identification of the protein affected by host-range mutation: Serological assays of fractions obtained in the ultracentrifuge permitted us to tentatively identify the protein component whose structure is believed to be altered by mutations within the h region of the T_2 genetic map.

Direction of Current Research: We are currently attempting to fractionate and isolate in pure form the proteins of the T_2 virus. When this is accomplished we plan to proceed with the elucidation of the effect of genetic mutations on protein structure and, eventually, with the comparison of fine structure genetic maps with maps of such alterations in protein structure.

If and when this comparison can be made we believe considerable insight into the mechanism by which genes control the process of protein synthesis will be gained.

Part B included Yes [ ] No [XX]

Form No. ORP-2
Oct. 1957

Serial No. NHI-48
1. Laboratory of Cellular Physiology and Metabolism
2. Cellular Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Structure of Ribonuclease and Its Correlation with Biological Activity.

Principal Investigators: Michael Sela and C. B. Anfinsen

Other Investigators: Frederick H. White, Jr. and William F. Harrington
Juanita P. Cooke (Technical)

Cooperating Units: None.

Man Years (Calendar year 1957):
Total: 1.67
Professional: .75
Other: .92

Patient Days (Calendar year 1957):
None.

Project Description: Elucidation of the chemical structure, size, shape and change of ribonuclease and some derivatives of RNase, and the correlation of these with changes in enzymatic activity. Out of these studies it should be possible to define the active groups and bonds in the molecule and thus to establish the "active center" of the enzyme. Some of the methods used should be of general interest in tackling similar problems concerning other biologically active proteins.

Progress During Past Year: Methods employed: Chemical modifications of ribonuclease were achieved by means of reduction with thioglycolic acid, followed by carboxymethylation with iodoacetic acid of the reduced material; oxidation with performic acid; esterification with methanol; carbobenzoxylation, as well as enzymatic digestion with pepsin, trypsin, subtilisin and carboxypeptidase. Physical properties, such as optical rotation, optical rotatory dispersion, viscosity, sedimentation, and ultraviolet absorption, of the various modified ribonucleases were measured and compared with those of native ribonuclease in water or in various solvents with strong hydrogen bond rupturing (urea, guanidine) or forming (saturated aqueous lithium bromide) properties.

The enzymatic activity of ribonuclease was followed by methods involving precipitation, dialysis or appearance of new acid groups during the digestion (pH stat). The activity of ribonuclease was followed as a function of salt, urea or guanidine concentration, as well as in the cases of the various chemical modifications.

Major Findings: A. Reduction and Alkylation as a Method for Breaking Disulfide Bridges in Proteins. (With Dr. White) A method has been developed for complete reduction of all the disulfide bridges in proteins, such as ribonuclease and lysozyme. The sulfhydryl groups formed were meshed selectively with carboxymethyl groups. The method seems of general interest as it does not attack tryptophan, an amino acid destroyed during the oxidative cleavage of disulfide bridges with performic acid. The extent of reduction can be followed either by absorption changes upon the reaction of the reduced protein with p-chloromercuribenzoate or by analysis of the amount of S-carboxymethyl-N-dinitrophenylcysteine in the hydrolyzate of the carboxymethylated protein.

B. Correlation of the Enzymatic Activity of Ribonuclease with the Extent of Reduction. (With Dr. White) During the reduction with thioglycolic acid in 8 M urea at pH 8.5, the activity of the enzyme decreases in a manner which indicates that certainly one, and perhaps two, of the four disulfide bridges are not essential for catalysis. Carboxymethylation of the sulfhydryl groups does not affect the enzyme activity of the intermediates. Regeneration of enzymatic activity takes place upon reoxidation with molecular oxygen.

C. Methylation of Ribonuclease. Reaction of ribonuclease with methanolic hydrochloric acid yields a material in which all the carboxyl groups in the molecule are esterified. The esterification is paralleled by a loss of catalytic activity of the enzyme. The fully esterified ribonuclease is completely inactive. Method used for deesterification (dilate alkali) did not regenerate the activity of the enzyme.

D. Reaction with Carboxypeptidase. Upon digestion of ribonuclease with carboxypeptidase in 6 M urea 52% of the C-terminal value was released, but only traces of serine and alanine. No detectable loss in ribonuclease activity was observed in samples so treated.

E. Correlation of Ribonuclease Activity with a Shift in Ultraviolet Spectrum. Various physical parameters of bovine pancreatic ribonuclease and of some of its derivatives prepared by oxidation or reduction of disulfide bridges, methylation and proteolytic digestion, have been investigated. Ultraviolet spectrophotometric measurements appear to establish a correlation between specific spectral properties of the materials and their enzymatic activity. Viscometric and optical rotatory studies, on the other hand, indicate that minor modifications in secondary structure may occur without detectable inactivation.

Polyvalent anions and polyanions almost completely prevent the unfolding and spectral shift effects of 8 M urea, and of guanidinium ions at concentration less than 3 M. These findings suggest that the full activity of ribonuclease in the presence of such denaturing agents, is due to a refolding of the protein under the influence of ribonucleic acid.

Direction of Current Research: Further studies will be carried out to help establish correlations between specific aspects of structure and the biological activity of the enzyme.

Part B included Yes [XX] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Sela, Michael, White, Frederick H., Jr., and Anfinsen, Christian B. Reductive cleavage of disulfide bridges in ribonuclease. Science, 125, 691-692 (1957).

Sela, Michael and Anfinsen, Christian B. Some spectrophotometric and polarimetric experiments with ribonuclease. Biochim. Biophys. Acta, 24, 229-239 (1957).

Sela, Michael, Anfinsen, Christian B. and Harrington, William F. The correlation of ribonuclease activity with specific aspects of tertiary structure. Biochim. Biophys. Acta, 26, 502-512 (1957).

Honors and Awards relating to this project: None.

Form No. ORP-2
Oct. 1957

Serial No. NHI-49
1. Laboratory of Cellular Physiology and Metabolism
2. Cellular Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Structure and Metabolism of Mucopolysaccharides.

Principal Investigator: Edward D. Korn

Other Investigators: Bertha Neal (Technical, October-December)

Cooperating Units: Dr. Karl Meyer, Columbia University, New York, New York.

Man Years (Calendar year 1957):
Total: 1.25
Professional: 1.0
Other: .25

Patient Days (Calendar year 1957):
None.

Project Description:

Objectives: To determine the structure of several mucopolysaccharides including heparin and chondroitin sulfates A, B, and C. To study the nature of the enzymatic degradation and synthesis of such compounds. To assess the possible biological activity of partial enzymatic hydrolysis products of heparin. To study the localization, properties and synthesis of heparin in mast cell tumors.

Methods Employed: Enzymic preparations are prepared from bacteria (Flavobacterium isolated in this laboratory) adapted to growth on one of several specific polysaccharides. These enzymes are then employed as analytical reagents for the degradation of the mucopolysaccharides to simpler oligosaccharides that are isolated by chromatographic techniques and characterized.

Mast cell tumors are homogenized and fractionated by centrifugation and the heparin content of each fraction determined by a sensitive method developed in this laboratory. The heparin is then solubilized by one of several means and the properties of the soluble material studied by physical, chemical and biological techniques.

Major Findings: 1. In collaboration with Dr. Karl Meyer of Columbia University, the following has been found:

a) Hyaluronic acid is degraded to a disaccharide glucuronyl-N-acetyl glucosamine unsaturated in the 4,5 position of the uronidic moiety. This product is identical to that produced by other bacterial enzymes.

b) Chondroitin sulfates A and C are degraded to the analogous sulfated Δ4,5-glucuronyl-N-acetyl glucosamine. In a secondary reaction this compound is desulfated. No other enzyme with this ability has been described.

c) Extracts of bacteria adapted to chondroitin sulfate B, in addition to the above two reaction, catalyze the degradation of chondroitin sulfate B to the same unsaturated disaccharide obtained from A and C. This indicates that the only difference between B and A and C is the uronic acid (iduronic in B and glucuronic in A and C) both of which yield the same Δ4,5 unsaturated uronidic group.

In addition to sulfatase activity, these extracts also contain enzymes which eventually split the unsaturated disaccharide to free N-acetyl galactosamine. This enzyme similarly degrades the unsaturated disaccharides (but not the saturated disaccharides) from chondroitin sulfates A and C to N-acetylgalactosamine.

d) Heparin and heparitin sulfate are both degraded by heparin-adapted bacterial extracts to a different unsaturated disaccharide than that obtained from hyaluronic acid. It differs in some way other than the presence of sulfate and absence of N-acetyl groups. Again there are secondary enzymic reactions which desulfate the disaccharide and split it to free glucosamine.

Neither heparin, heparitin sulfate nor chondroitin sulfate B are degraded by these enzymes if they are first chemically desulfated.

2. a) A new procedure has been developed for the isolation of heparin. It involved the enzymic removal (when necessary) of proteins and lipids with crude pancreatin, the precipitation of heparin from 1 M NaCl with a quaternary amine (cetyltrimethylammonium bromide) and the removal of the amino with KCNS. This method is extremely mild and is quantitative at the level of a fraction of a milligram. The heparin can then be determined by chemical or biological assay.

b) It has been found that essentially all of the heparin in mast cell tumor (mouse) homogenates is sedimentable. About 16% sediments when centrifuged for 10 minutes at 50 x g; about 60% at 10,000 x g for 10 minutes; and about 10% at 100,000 x g for one hour; about 10% is left in the final supernatant solution.

c) Heparin can be released from the sedimentable particles by ultrasonic vibration or, more simply, lysis by one or another means. It is then not sedimentable at 100,000 x g for 1 hour.

Proposed Course of Project: 1. With the active participation of Dr. Karl Meyer and his associates the separation and identification of the partial enzymatic digestion products of heparin and the chondroitin sulfates will be continued.

2. The solubilized heparin from mast cell tumors will be further studied to see if it differs from heparin prepared by more severe methods in its chemical, physical and biological properties. If it is obtainable complexed to protein, this complex will be studied.

3. Some experiments will be initiated on the biosynthesis of heparin by mast cell tumor, in vitro.

Part B included Yes [XX] No [ ]

Serial No. NHI-49

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Korn, Edward D. The degradation of heparin by bacterial enzymes. III. A comparison of the degradation of heparin, hyaluronic acid and chondroitin sulfate. J. Biol. Chem., 226, 841-844 (1957).

Linker, A., Hoffman, P., Sampson, P., Meyer, K., and Korn, Edward D., The degradation of hyaluronic acid, the chondroitin sulfates and heparin by bacterial enzymes (Flavobacterium). Biochim. et Biophys. Acta, 25, 658-659 (1957).

Honors and Awards relating to this project: None.

Form No. ORP-2
Oct. 1957

Serial No. NHI-50
1. Laboratory of Cellular Physiology and Metabolism
2. Cellular Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on Lipoproteins.

Principal Investigators: Martin Rodbell

Other Investigators: Thomas W. Quigley, Jr., (Technical, January-June)
Edward D. Korn

Cooperating Units: None

Man Years (Calendar year 1957):
Total: 1.5
Professional: 1.0
Other: .5

Patient Days (Calendar year 1957):
None.

Project Description:

Objectives: To study the structure, function and metabolism of lipoproteins with special emphasis on the role of plasma lipoproteins in fat transport.

Methods Employed: Lipoproteins are isolated by ultracentrifugation and other techniques. The lipid composition is determined by chemical analysis after delipidation by chloroform-methanol or alcohol-acetone. The proteins are partially characterized by determination of the N- and C-terminal amino acids. The lipoproteins are subjected to enzymic digestion by lipoprotein lipase and several phospholipases.

Major Findings: 1. a. Incubation of lipoproteins with either phospholipase A or C destroys the ability of the lipoprotein to activate triglycerides for hydrolysis by lipoprotein lipase. The phospholipid, therefore, seems essential.

b. When digested with phospholipase A, which removes the α-fatty acid and forms lysolecithin, the lipoproteins stay in solution and the lipid-protein complex is not destroyed. With phospholipase C, which removes phosphoryl choline thus forming a diglyceride, the lipoprotein is completely denatured and both the protein and lipid come out of solution.

c. The rate of hydrolysis of lipoprotein phospholipid is much greater than phospholipid emulsions with both phospholipases. This is analogous to what was found with lipoprotein lipase.

2. The human plasma lipoproteins have been separated ultracentrifugally into seven fractions of increasing density (increasing percentage of protein, decreasing percentage of triglyceride) from chylomicrons to ghe highest density lipoprotein. N-terminal amino acids were determined on the protein moieties after delipidation with organic solvents.

a. Chylomicrons and the fraction of next lowest density both contained predominantly N-terminal threonine but had quite significant concentrations of N-terminal serine and aspartic acid as well. The next two fractions (Sf 50-100 and 20-50) had essentially equal amounts of N-terminal aspartic acid, threonine, serine and glutamic acid. The next two fractions (Sf 10-17 and 3-8) were predominantly N-terminal glutamic acid with serine and threonine also present but no aspartic acid. The last fraction contained mainly N-terminal aspartic acid with serine and threonine also present but no glutamic acid.

Chyle chylomicrons were also analyzed and they were predominantly N-terminal aspartic acid with serine and threonine also present.

b. Because of their differing molar ratios the various N-terminal amino acids appear to represent protein moieties which are not linked to each other on one molecular species. This would imply that a very complex situation may exist with overlapping density spectra of at least four lipoproteins each of which may or may not be involved in fat transport and metabolism.

c. The yields of N-terminal aspartic acid from the highest density lipoprotein indicate a molecular weight of about 50,000. This is confirmed by similar results on dog plasma lipoprotein which gave a value of 45,000. This is one-half the molecular weight indicated by other workers.

Proposed Course of Project: 1. a. New techniques, probably chromatographic, must be developed to obtain each of the several proteins represented by the different N-terminal amino acids free from the others. It will then be possible to determine if all are indeed lipoproteins or if some represent contaminant non-lipoprotein molecules. These molecules can then be analyzed individually to see of they differ with respect to lipids and if all the proteins with the same N-terminal amino acid are identical.

2. Parallel experiments will be done with dog (and other species) plasma to obtain a comparative picture and so that metabolic experiments can be performed with labelled lipoproteins to see which of the several types are truly involved in lipid metabolism.

Part B included Yes [XX] No [ ]

Serial No. NHI-50

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Korn, E.D. and Quigley, T.W. On the nature of lipoprotein lipase. Third International Conference on the Biochemical Problems of Lipids, Brussels, in press.

Korn, E.D. Lipoprotein lipase. Chemistry of Lipids Symposium, Cleveland, May, 1957, in press.

Korn, E.D. Inactivation of lipoprotein lipase by heparinase. J. Biol. Chem., 226, 827-832 (1957).

Korn, E.D. and Quigley, T.W. Lipoprotein lipase from chicken adipose tissue. J. Biol. Chem., 226, 833-839 (1957).

Honors and Awards relating to this project:

E. D. Korn - Invited speaker at the Symposium on Chemistry of Lipids as related to Atherosclerosis, Cleveland, May, 1957.

Form No. ORP-2
Oct. 1957

Serial No. NHI-51
1. Laboratory of Cellular Physiology and Metabolism
2. Cellular Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: A Study of the Mechanism of Protein Biosynthesis in the Hen Oviduct.

Principal Investigator: Richard W. Hendler

Other Investigators: None.

Cooperating Units: Dr. Albert J. Dalton of the Cancer Institute Serial No._____, and Dr. George G. Glenner of the Institute of Arthritis and Metabolic Diseases Serial No._____, and Dr. E. A. Peterson of the Cancer Institute Serial No._____.

Man Years (Calendar year 1957):
Total: 1.0
Professional: 1.0
Other: None.

Patient Days (Calendar year 1957):
None.

Project Description: 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.

Progress During Past Year: Previous kinetic studies on the incorporation of amino acids into the proteins of hen oviduct indicated that the amino acids first passed through the cell-debris material before appearing in the soluble protein fraction. A study of the fine structure of the cells and of the cell-debris indicated that the ergastoplasm (microsomal material) formed a lining for sacs which appeared to contain the secretory material, and be the site for the initial incorporation of radioactive amino acid within the cell-debris. This idea was supported by the observation that a washed cell-debris preparation, pre-labeled with radioactive amino acid released into buffer upon subsequent incubation proteins of higher specific activity than the cell debris itself or of the corresponding supernatant proteins at the end of the preincubation. A collaborative effort with Dr. E. A. Peterson of the National Cancer Institute, employing the sensitive technique

of cellulose column chromatography established the identity of the radioactive protein released from the cell debris with the normally occurring soluble proteins of this tissue. A further characterization of the soluble proteins of this tissue was also obtained. The effects of various stimulators and inhibitors on the system was studied as well as varying the conditions of incubation. The findings in the project discussed above were submitted in the form of a paper for publication in the Journal of Biological Chemistry.

The hen oviduct, when homogenized, essentially loses its ability to incorporate free amino acids into its proteins. However, if the tissue is labeled with radioactive amino acid while the cells are intact, and then homogenized, subsequent incubation leads to an increase in the total amount of radioactivity in the proteins. Suitable controls show that free amino acid is practically inert. This suggests that there is a compound between free amino acid and protein in its complexity which accumulates in the cells during the whole cell incubation and which serves as a precursor for the proteins. The site and nature of this proposed intermediate are now under investigation.

Direction of Current Research: Attempts will be made to localize cytologically and chemically the "intermediate" indicated by the investigations discussed above. If possible, the exact chemical structure and mode of action will be investigated.

Part B included Yes /XX/ No /__/

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Hendler, Richard W., Dalton, Albert J. and Glenner, George G. A cytological study of the albumin-secreting cells of the hen oviduct. J. Biophysic. and Biochem. Cytol. 3, 325-330 (1957).

Hendler, Richard W. Evidence for an intermediate stage in the process of amino acid incorporation into hen oviduct proteins. Biochim. et Biophys. Acta 25, 444-445 (1957).

The following paper has been published since the last report on the project of the same title. As was mentioned in the last progress report, this project has been temporarily terminated to allow full time to be spent on the protein biosynthesis project.

Hendler, Richard W. A study of carbon dioxide fixation in the hen oviduct. Biochim. et Biophys. Acta, 24, 187-192 (1957).

Form No. ORP-2
Oct. 1957

Serial No. NHI-52
1. Laboratory of Cellular Physiology and Metabolism
2. Cellular Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Structural Role of Proline and Hydroxyproline in Proteins.

Principal Investigators: William F. Harrington and Michael Sela

Other Investigators: A. P. Pfuderer (summer)

Cooperating Units: None

Man Years (Calendar year 1957):
Total: 1.5
Professional: 1.25
Other: .25

Patient Days (Calendar year 1957):
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.

Progress During Past Year: The properties of the proline residue in the polypeptide chain have been investigated through the use of polymers of this imino acid which have been recently synthesized at the Weizmann Institute, Israel. The configuration of poly-l-proline polymers in solution have been studied primarily by means of various physical methods such as optical rotation, viscosity and "sedimentation".

Major Findings: 1. Poly-l-proline exists in water as a helical molecule with peptide bonds in the trans configuration.

2. In various solvents of low water activity it has been found that the helical pattern of poly-l-proline is destroyed and the evidence suggests that this transition is brought about through a series of trans-cis isomerizations at the peptide bonds. These observations are consistent with the view that trans-cis isomerizations may occur in protein molecules under conditions such as a favorable neighboring amino acid sequence or hydrophobic region of the protein.

3. Preliminary studies on collagen and gelatin which contain high percentages of proline and hydroxyproline show similar optical rotatory changes. These findings are being examined with a view toward the possibility that the peptide bond configuration of these residues is of primary importance in the proposed three-stranded structure of collagen.

Direction of Current Research: 1. Studies on collagens and gelatins from various sources and with differing proline and hydroxyproline content will be continued.

2. In collaboration with members of the Weizmann Institute, Israel, various model polymers and copolymers of proline and hydroxyproline will be examined. Optical rotatory sedimentation, viscosity, light scattering, flow birefringence and osmometric properties will be measured and correlated with the configuration of these model chains in solution. It is hoped that this information will allow the unquestioned importance of proline in protein structures to be clarified.

Part B included Yes /XX/ No /_/

Serial No. NHI-52

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Harrington, William F. and Sela, Michael. Studies on the structure of poly-l-proline in solution. Biochim. et Biophys. Acta, in press.

Honors and Awards relating to this project:

None.

Form No. CRP-2
Oct. 1957

Serial No. NHI-53
1. Laboratory of Cellular Physiology and Metabolism
2. Cellular Physiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Investigation of Structural and Functional Relationships in Ribonuclease.

Principal Investigator: Frederick H. White, Jr.

Other Investigators: Christian B. Anfinsen and Michael Sela

Cooperating Units: None.

Man Years (Calendar year 1957):
Total: 1.6
Professional: 1.6
Other: None.

Patient Days (Calendar year 1957):
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.

Progress During the Past Year: Reduced, carboxymethylated ribonuclease (obtained by reduction of native ribonuclease with thioglycolic acid and carboxymethylation of the resulting sulfhydryl groups with iodoacetic acid) is purified by chromatography. The reductively cleaved disulfide bridges in this enzyme are then located by enzymatic degradation followed by isolation and identification of peptides containing S-carboxymethyl-cysteine. Enzymatic activity (measured by standard methods involving spectrophotometry) may then be associated with specific disulfide bridges.

Major Findings: A. Activity of reduced ribonuclease has been correlated with the extent of reduction (determined by spectrophotometric titration with p-chloromercuribenzoate).

B. Additional procedures have been successfully employed for determination of the extent of reduction in reduced, carboxymethylated ribonuclease. They are (1) analysis for S-carboxymethylcysteine, and (2) analysis for cysteine.

C. Partially reduced, carboxymethylated ribonuclease has been subjected to chromatographic studies, and several components have been isolated. All of these components possess ribonuclease activity.

D. The presence of β-carboxy β-aminoethyl carboxymethyl disulfide (CACD) in partially reduced ribonuclease has been proven, thus revealing a complicating factor in the reduction of protein disulfide bridges.

Direction of Current Research: A. It is planned to employ further tests for the homogeneity of the isolated components of partially reduced ribonuclease and ultimately to locate the reductively cleaved disulfide bridges of these components.

B. Experiments are in progress to establish a quantitative correlation between CACD content of reduced ribonuclease and the extent of reduction.

Part B included Yes /XX/ No /_/

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Sela, Michael, White, Frederick H., Jr., and Anfinsen, Christian B. Reductive cleavage of disulfide bridges in ribonuclease. Science, 125, 691-692 (1957).

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
Metabolism Section

Estimated Obligations for FY 1958

Total:	$756,075
Direct:	$271,730
Reimbursements:	484,345

Form No. ORP-2
Oct. 1957

Serial No. NHI-54
1. Laboratory of Cellular Physiology and Metabolism
2. Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Energy Transfer Associated with Electron Transport.

Principal Investigators: W. Wayne Kielley and J. Ramsey Bronk

Other Investigators: Lisa Barnett (Technical) and Clarence Edwards (Technical - summer)

Cooperating Units: None.

Man Years (Calendar year 1957):
Total: 3.25
Professional: 2.0
Other: 1.25

Patient Days (Calendar year 1957):
None.

Project Description: Progress toward the elucidation of the mechanism of electron transport phosphorylation has been greatly speeded by the recent development in several laboratories of procedures for fragmenting mitochondria to give submitochondrial particles capable of catalyzing oxidative phosphorylation. The submitochondrial phosphorylation system developed in this laboratory has lost many of the activities usually associated with intact mitochondria but in addition to phosphorylation associated with the oxidation of either succinate of reduced pyridine nucleotide (DPNH) these particles will catalyze the exchange of inorganic phosphate with the terminal phosphate of adenosinetriphosphate (ATP) and the exchange of adenosinediphosphate (ADP) with the ADP moiety of ATP. The fragments also possess an active ATPase. If the continued association of the two exchange reactions and ATPase activity with phosphorylation in the submitochondrial fragments can be shown to be due to the identity of these activities with the mechanism of phosphorylation, considerable further insight into the nature of the terminal steps of the phosphorylation process will have been gained. Our recent activity has been devoted to studying the properties of the phosphorylation and the two exchange reactions in this preparation, and with the study of the relationship of these three processes with each other and with the the associated ATPase activity.

Progress During Past Year: A careful study of the conditions and medium employed for the disintegration of mitochondria in the sonic oscillator led to the development of a more satisfactory suspension medium which allowed shorter disintegration time to be employed. This change in technique resulted in an improvement in the activity of the preparation and clear demonstration of the steps of phosphorylation catalyzed by this particle preparation. All of the phosphorylation was found to be associated with oxidation steps leading to the reduction of cytochrome c. When reduced cytochrome c was used as an electron donor there was little or no phosphate uptake associated with its oxidation. This preparation will oxidize succinate with P/O ratios of 0.6 - 0.95 and DPNH with P/O ratios of 0.7 - 1.3. Although the preparation contains no bound diphosphopyridine nucleotide (DPN^+), it will oxidize β-OH butyrate if DPN^+ is added giving P/O ratios of 1.0 - 1.5. Essentially the same P/O ratios were found using either oxygen or cytochrome c as electron acceptors, indicating that for the steps of phosphorylation that are operating an efficiency of 60-95% can be obtained. This compares favorably with efficiencies found with intact mitochondria.

The phosphorylation and ATPase activities of the preparation as well as the exchange of ADP and inorganic phosphate with their respective moieties of ATP are all dependent on the presence of magnesium ions. However, it has been found that three other divalent ions (Mn^{++}, Co^{++} and Fe^{++}) can substitute for magnesium as activators of all four processes.

2,4-dinitrophenol (DNP) will suppress phosphorylation and the exchange of inorganic phosphate with the terminal phosphate of ATP. However, a study of the dependence of the four processes on temperature yielded the information that DNP will inhibit the exchange of ADP with the ADP moiety of ATP only at temperatures above 15°C and also that DNP accelerates ATPase only above 15°C. This information, together with the fact that a higher concentration of DNP was necessary to produce complete uncoupling of phosphorylation or complete inhibition of inorganic phosphate exchange at 43°C than was necessary at 28°C has led us to propose that if the exchange reactions and ATPase are associated with the phosphorylation process then they must be related by a mechanism analogous to the following:

$$(1)\quad ATP + E \rightleftarrows ADP + E \sim P$$

$$(2)\quad E \sim P \rightleftarrows E^* + Pi$$

$$(3)\quad E^* \rightarrow E + \text{energy}$$

Reaction (1) represents the ADP exchange, reactions (1) and (2) the inorganic phosphate exchange and the sum of all three reactions, ATPase activity. Net phosphorylation could take place if a high energy intermediate (E^*) was supplied to the system by oxidative reactions. The fact that the ADP exchange cannot be completely inhibited by concentrations of DNP that will completely inhibit the exchange of inorganic phosphate means that the exchange of ADP rather than the exchange of

inorganic phosphate must take place in reaction 1. The over-all ATPase activity of the system leads us to modify this reaction scheme with the suggestion that some of the ATPase activity of the submitochondrial preparation is unrelated to the exchange or phosphorylation processes.

Direction of Current Research: Present efforts are concentrated along three lines. One is an attempt to obtain independent evidence that the two exchange reactions and at least some of the ATPase activity are related to the phosphorylation process. This is necessary in order to see whether the mechanism proposed above for the exchange reactions (steps 1 and 2) can represent the terminal steps of the phosphorylation process.

Secondly, further attempts will be made to elucidate the central problem of the phosphorylation process, namely, how the high energy intermediate is produced by the oxidative reactions and what form it takes. In this connection fractionation of the system will be attempted together with extensive spectrophotometric study of the preparation and any fragments obtained from it.

The third approach comes as a result of the finding that thyroxin or triiodothyronine can under certain circumstances influence the efficiency of phosphorylation in the submitochondrial fragment preparation. The effects of thyroxin on this system are thus being studied to determine whether they represent a possible mechanism for control of energy yielding reactions in the intact animal.

Part B included Yes /XX/ No /_/

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Kielley, W. Wayne and Bronk, J. Ramsey. Oxidative phosphorylation in sonic extracts of rat liver mitochondria. Biochim. Biophys. Acta, 23, 440-449 (1957).

Bronk, J. Ramsey and Kielley, W. Wayne. Ionic requirements for oxidative phosphorylation, ATP-P^{32} exchange and ATPase. Biochim. Biophys. Acta, 24, 440-441 (1957).

Kielley, W. Wayne and Bronk, J. Ramsey. Oxidative phosphorylation in mitochondrial fragments obtained by sonic vibration. J. Biol. Chem., in press.

Honors and Awards relating to this project: None.

Form No. ORP-2
Oct. 1957

Serial No. NHI-55

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the Mechanism of Action of the Sulfonylurea Hypoglycemic Agents. (Began January, 1956 - Ended February, 1957).

Principal Investigator: Martha Vaughan

Other Investigator: Jane Logan (Technician)

Cooperating units: None

Man Years (calendar year 1957)
Total: .1
Professional: .05
Other: .05

Patient days: None

Project Description:

General Purpose: Two similar sulfonylurea derivatives which cause hypoglycemia have recently been employed in the treatment of diabetes mellitus. It is of interest both from a therapeutic and from a theoretical point of view to ascertain the mechanism of their hypoglycemic action.

Progress: A few experiments were done to determine the effect of Orinase on the formation of active phosphorylase by liver slices incubated in vitro. In previous studies Orinase caused a marked inhibition of the epinephrine and glucagon effects on release by liver slices and it was felt that these observations might be explained by a drug induced increase of active phosphorylase in liver slices.

Part B. included Yes X

Part B. Honors, Awards and Publications

Publications

1. Vaughan, M. Studies on the Mechanism of Action of Orinase (Tolbutamide) J. Am. Diabetes Assn. 6, 16-18, 1957.

2. Vaughan, M. The Effect of Tolbutamide on Glucose Production by the Liver in Vitro. Annals. N. Y. Acad. Sci. 71, 112-17, 1957.

Form No. ORP-2
Oct. 1957

Serial No. NHI-56 (c)

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the Mechanism of Action of Dietary Fats in Relation to Serum Cholesterol (started May, 1957; will continue at least another year)

Principal Investigators: Joel Avigan and Daniel Steinberg

Other Investigators: Mary Ann Hurley, Hugh Vroman (Technicians).

Cooperating units: None

Man Years: (calendar year 1957) Patient days: 240
Total: 1.7
Professional: .7
Other: 1.

Project Description:

General Purpose of Research: To determine in what ways the nature of dietary fat influences lipid metabolism, particularly the levels of serum cholesterol.

Progress During Past Year: The possibility that unsaturated fats in the diet might suppress cholesterol synthesis was explored using rats as experimental animals. Contrary to expectation, the rate of hepatic synthesis of cholesterol measured both with C^{14} acetate and with T_2O was increased rather than decreased. The serum cholesterol level in rats receiving corn oil (20% of the diet by weight) was very slightly but significantly lower than that in animals receiving equal amounts of coconut oil but not significantly different from controls on a low fat diet.

In view of this negative result in rats and in view of the small effect on serum cholesterol further studies were shifted to the clinic and the degradative rather than the synthetic rate was investigated. Patients were placed on liquid formula diets containing corn oil or coconut oil in an amount sufficient to represent 60% of the total caloric intake. Tritium labeled cholesterol was given and the disappearance from serum followed for a period of a week or two prior to and after this shift of diet from one oil to the other. Despite the shift in diet there was no apparent break in the curve for cholesterol disappearance. Attempts to obtain an alternative measure of the rate of cholesterol degradation in terms of release of tritium to the body water pool proved impracticable.

Direction of Current Research: Additional studies of cholesterol degradation are in progress and a method for direct determination of fecal bile acids and fecal steroids is being used to obtain further information about the effects of dietary fats on cholesterol metabolism.

Part B. included Yes X

Part B. Honors, Awards and Publications

Publications:

1. Avigan, J., Eder, H. A. and Steinberg, D. Metabolism of the Protein Moiety of Rabbit Serum Lipoproteins. Proc. Soc. Exper. Biol. and Med. 95, 429-33, 1957.

Form No. ORP-2
Oct. 1957

Serial No. NHI-57 (c)

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part. A.

Project Title: Studies of Inhibitors of Cholesterol Biosynthesis (Started in August, 1955)

Principal Investigators: Daniel Steinberg, Donald S. Fredrickson, Joel Avigan, R. Masters

Other Investigators: Jane Logan, Katsuto Ono

Cooperating units: None

Man Years (calendar year 1957)
Total: 1.95
Professional: 1.75
Other: .2

Patient days: 1500

Project Description:

Progress during past year: It has now been firmly established that the dramatic effects of Δ^4-cholestenone on the adrenal gland are associated with a marked inhibition of the output of steroid hormone from it. The cholesterol and ascorbic acid content of the glands are found to be low; the salt retaining capacity of the treated rats is low; despite the six fold increase in adrenal weight there is no involution of the thymus; and now it has been shown that adrenal vein blood in the treated animals contains only one-sixth the amount of compound B found in control animals (compound B constitutes about 95% of the steroid output of the rat adrenal). These findings could be of great importance in clarifying the relationship of cholesterol metabolism to steroid synthesis. For example, we have shown that feeding cholesterol partially protects against the toxic effects of Δ^4-cholestenone. It has now been shown that this is not due to the interference with absorption of Δ^4-cholestenone. This protective effect suggests that exogenous cholesterol can be used to maintain normal steroid output. The production of adrenal insufficiency by an inhibitor of cholesterol biosynthesis suggests that steroid synthesis goes by way of cholesterol to a large extent at least. Previous studies have left in doubt the question of whether cholesterol was merely a potential precursor, or rather an obligatory precursor, for adrenal steroids.

Clinical studies in several patients were carried out at dose levels very low compared to those used in animals. Even at this low dosage (70 mg/kg/day) these patients accumulated dihydro-cholesterol in their serum (up to several hundred mg. %) and several of them showed an elevated BSP retention. Clinical studies have been discontinued.

Δ^4-sitostenone and 3-chloro-cholesterol have been tested and found ineffectual in inhibiting cholesterol synthesis in rats.

General Purpose of Research: To investigate the mechanisms controlling cholesterol biosynthesis and to find a metabolic inhibitor suitable for clinical use in the treatment of hyper-cholesterolemia.

Direction of Current Research: A series of structural analogues of cholesterol and cholestenone have been made available to us through the cooperation of Merck, Sharp and Dohme, and Upjohn. These compounds will be tested as inhibitors in the hope of finding an inhibitor which does not lead to end product accumulation.

The adrenal effects of cholesterol will be studied further since it may be possible to obtain fundamental information about the mechanism of adrenal steroid synthesis in relation to cholesterol metabolism.

Part B. included Yes X

Part B. Honors, Awards and Publications

Publications:

1. Fredrickson, D. S. and Steinberg, D. Failure of Alpha-Phenylbutyrate and Beta-Phenylvalerate in Treatment of Hypercholesterolemia. Circulation 15, 391-96, 1957.

2. Masters, R. and Steinberg, D. Studies on the Mechanism of Action of Alpha-Phenylbutyrate. Biochim. et Biophys. Acta. (In Press).

Form No. ORP-2
Oct. 1957

Serial No. NHI-58

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: High molecular weight components of rabbit skeletal muscle and their interactions.

Principal Investigator: Elemer Mihalyi

Other Investigators: M. I. Knoller (Technician)

Cooperating units: The studies on the ribonucleic acid were done in collaboration with Dr. Dan F. Bradley of the National Institute of Mental Health.

Man Years (calendar year 1957)
Total: 1.
Professional: .6
Other: .4

Patient days: None

Project Description:

Objectives: To study the extraction of ribonucleic acid from rabbit muscle, its relation to an accompanying protein and its physiochemical and chemical characteristics.

Methods: Chemical, molecular kinetic and optical analysis of the materials. Separation by column fractionation and electrophoresis.

Major findings: Myosin B isolated from rabbit muscle by standard methods contains up to 1% ribonucleic acid as an impurity. Methods involving denaturation of the myosin yield a product with a protein/RNA ratio of 1.3-1.4, similar in RNA content to fish nucleo-tropomyosins. The protein is not myosin B, but rather a heat stable protein with a low aromatic amino acid content, similar to tropomyosin. The RNA is not tightly bound to this protein, however, as they can be further separated by electrophoresis or by chromatography on Ecteola columns, yielding a product with a protein/RNA ratio of less than 0.1. The mean nucleotide composition of the RNA is 18.3% adenylic acid, 31.5% Guanylic acid, 30.9% cytidylic acid and 18.9% uridylic acid. All samples analyzed were of this same composition despite wide variations in the extent and methods of fractionation of the RNA. The RNAs were electrophoretically homogeneous but highly heterogeneous in the ultra-centrifuge, having a concentration independent, mean sedimentation coefficient of 6.6 S. The preparations showed a typical RNA ultra-

violet absorption spectrum. The absorption increased upon base hydrolysis and the spectrum of the hydrolysate coincided with the sum of the absorptions of the component nucleotides as calculated from the measured nucleotide composition. The differential spectrum, native vs. base hydrolyzed, has two maxima, which nearly coincide with those of guanylic and cytidylic acids.

Significance to Heart Research: Very likely there is no basic difference between heart and skeletal muscle. Investigations on the more easily obtainable rabbit skeletal muscle will help us to understand the function of the heart in health and disease.

Proposed course of project: Further characterization of the high molecular weight components of rabbit skeletal muscle and similar investigations on heart muscle.

Part B. included Yes X

Part B. Honors, Awards and Publications

Publications:

1. Mihalyi, E., Laki, K., Knoller, M. I. Nucleic acid and nucleotide content of myosin preparations. Arch. Biochem and Biophys 68, 130-143, 1957.

2. Mihalyi, E., Bradley, D. F. and Knoller, M. I. Some physical and chemical properties of the ribonucleic acid contaminant of rabbit muscle myosin preparations. J. Am. Chem. Soc. (In Press) To appear in December, 1957.

3. Steinberg, D. and Mihalyi, E. The Chemistry of Proteins. Ann. Rev. Biochem. 26, 373-418, 1957.

Form No. ORP-2
Oct. 1957

Serial No. NHI-59

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on Experimental Nephritis: The Nephrotoxic Serum Antigen.

Principal Investigator: James H. Baxter

Other Investigators: Howard Goodman, James Allen and Patricia Duffy

Cooperating units: None

Man Years (calendar year 1957)
Total: 1.7
Professional: 1.2
Other: .5

Patient days: None

Project Description:

Objectives: To purify and characterize the antigen which is present in kidney and other tissues of the rat, which will induce the formation in rabbits of antiserum nephrotoxic for the rat. Also to purify and characterize the soluble protective factor derived from the antigen.

Methods: The nephrotoxic serum antigen is extracted from rat kidney or rat lung homogenate. Soluble protective factor is produced by tryptic digestion of the tissue homogenates. The antigen or soluble protective factor are identified and quantitated by their ability to neutralize the proteinuria-producing effect of a standard dose of nephrotoxic antiserum. These techniques have previously been described in detail. Methods utilized in attempts to purify the factors have included dialysis and lyophilization followed by free and zone electrophoresis, alcohol precipitation, or ultracentrifugation.

Progress: Much colored material and material containing nucleic acid migrate rapidly at pH 8.6 and can be eliminated by zone electrophoresis. However, the active material is spread throughout a wide, skewed protein peak and it has not been possible to elute a fraction with considerably increased ratio of activity: protein. Likewise, in preliminary experiments by Dr. Allen, the antigen activity has been spread through the series of precipitates obtained with alcohol.

The preparation containing soluble protective factor has been examined in the analytical ultracentrifuge. Three peaks with uncorrected S values of 5.0, 3.6 and 2.0, respectively, together with a spread of slower colored material, were observed. Initial preparative runs suggest that the protective factor is associated with either the slower peak or the still slower material and that progress toward purification may be made by a combination of ultracentrifugation followed by electrophoresis of the material remaining in the upper halves of the centrifuge tubes, after the faster components have been centrifuged into the bottoms.

Direction of Current Research: As indicated above. If the protective factor can be purified it will be used in animal experiments. Attempts will then be made to prepare similar material from human kidneys.

Part B. included No X

Form No. ORP-2
Oct. 1957

Serial No. NHI-60 (c)

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on Lipoprotein Metabolism in Patients with Nephrosis.

Principal Investigator: James H. Baxter

Other Investigators: Howard Goodman and Patricia Duffy. Some of the studies have been done in collaboration with Joseph Bragdon and Robert Gordon.

Cooperating units: None

Man Years (calendar year 1957)
Total: 0.8
Professional: 0.4
Other: 0.4

Patient days: 350

Project Description:

Objectives: a) To characterize the serum lipoprotein abnormalities in nephrosis, and b) to determine the role of hypoalbuminemia in the causation of the lipoprotein disturbance.

Methods Employed: Lipid determinations have been done on patients' sera and on lipoprotein fractions separated from the sera by ultracentrifugation at various densities. Serum albumin has then been infused into some of the patients for periods of several weeks and the changes in serum lipids and lipoproteins in relation to the serum albumin levels have been noted. Levels of unesterified fatty acids have also been followed and correlated with albumin and lipoprotein levels.

Progress: a) It has been observed that not all patients with nephrosis have the same abnormal lipoprotein pattern. The patterns probably form a continuous spectrum but for convenience the patients may be divided into three groups: 1) those with marked elevation of the Sf $>$ 10 fraction, characterized by great elevation of triglycerides and very milky serum, 2) those with elevation of both Sf $>$10 and Sf 0-10 fractions, characterized by elevation of cholesterol and triglycerides in approximately normal proportions and serum of

moderate turbidity, and 3) those with elevation of the Sf 0-10 fraction alone, characterized by elevation of cholesterol (and phospholipid) without change in triglycerides, and by clear serum. There has been some suggestion that the first pattern is usually associated with the lowest levels of serum albumin. The pattern in a given patient may change from one time to another.

b) Infusions of serum albumin in quantities sufficient to raise the serum albumin level significantly for periods of several weeks have resulted in striking reduction of elevated serum lipid and lipoprotein levels. The changes have been much greater than could be accounted for by dilution due to expansion of plasma volume. Studies of the lipoprotein fractions indicate that albumin may bring about a reduction in both the SF $>$ 10 and the Sf 0-10 fractions when they are elevated. The mechanism of the albumin effect is not known. It is known that unesterified fatty acids are transported largely in combination with albumin. It is conceivable that a disturbance in this transport mechanism leads to the lipoprotein disturbance. Markedly elevated molar ratios of UFA/albumin have been noted in two nephrotics with milky serum. Another possibility is that the stimulus which results in the lipoprotein disturbance is a result of reduced oncotic effect of serum proteins.

Direction of Current Research: This project as originally planned is almost completed. It seems desirable to determine whether a substance which does not bind fatty acids, such as dextran, is like albumin capable of bringing about a reduction in abnormal lipoprotein levels. It is also desirable to determine whether the lipoproteins, in addition to albumin, can bind unesterified fatty acids, and something of the nature of the binding if it occurs.

Part B. included Yes X

Part B. Honors, Awards and Publications

Publications:

1. Baxter, James H. Lipoprotein Patterns and the Effects of Albumin Infusions in Nephrosis - Proceedings Ninth Annual Conference on the Nephrotic Syndrome (In Press).

Form No. ORP-2
Oct. 1957

Serial No. NHI-61 (c)

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar year 1957

Part A.

Project Title: Metabolism of Plasma Unesterified Fatty Acids (UFA)

Principal Investigator: Robert S. Gordon, Jr.

Other Investigators: Donald S. Fredrickson
Amelai Cherkes (Technician)

Cooperating units: None

Man Years (calendar year 1957)
Total: 1.75
Professional: .75
Other: 1.

Patient days: 750

Project Description:

Progress: Investigation of the turnover of plasma UFA and its conversion to CO_2, utilizing isotopic tracers, has given an understanding of the turnover, distribution, and metabolism of this material. The carbon dioxide produced by fasting human subjects is derived primarily from the oxidation of plasma UFA. In addition, this plasma lipid has been shown to be the metabolic precursor of the ketone bodies produced in diabetic acidosis.

Current Research: The present lines of investigation will be pursued with a view to determining possible differences in the rate of metabolism of UFA's in a variety of disease states. Possible differences in the metabolic fate of saturated as opposed to unsaturated fatty acids will be determined. The relationship of these data to data currently being collected on the metabolism of other lipids will be evaluated.

Part B. included Yes X

Part B. Honors, Awards, and Publications

Publications:

1. Gordon, R. S., Jr., Cherkes, A., and Gates, H. Unesterified Fatty Acid in Human Blood Plasma. II. The Transport Function of Unesterified Fatty Acid. J. Clin. Invest. 36, 810-15, 1957.

2. Brasdon, J. S., Havel, R. J. and Gordon, R. S., Jr. Effects of Carbohydrate Feeding on Serum Lipids and Lipoproteins in the Rat. Amer. J. of Physiol. 189, 63-67, 1957.

3. Fredrickson, D. S., and Gordon, R. S., Jr. The Metabolism of Unesterified Fatty Acids in Man. Symposium: Biochemical Problems of Lipids, Oxford, 1957. (In Press).

4. Gordon, R. S., Jr. $C^{14}O_2$ Excretion after the Intravenous Administration of Albumin-Bound Palmitate-1-C^{14} to Intact Rats. Arch. Biochem. & Biophys. (In Press).

Form No. ORP-2
Oct. 1957

Serial No. NHI-62 (c)

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Treatment of the Nephrotic Syndrome

Principal Investigator: Howard Goodman and James Baxter

Other Investigators: Patricia Duffy and Robert Bowser

Cooperating units: None

Man Years (calendar year 1957) Patient days: 500
Total: 0.5
Professional: 0.4
Other: 0.1

Project Description:

Progress: Studies on the steroid treatment of patients with idiopathic nephrotic syndrome were initiated shortly after the opening of the Clinical Center and have continued to the present date in conjunction with other studies (i.e. lipid metabolism), in the same patients. These studies are fully reported in the two publications cited below. In summary, treatment with 40 mg. of prednisone of 160 mg. hydrocortisone daily was continued for three weeks to one month, or until maximum obtainable fall in proteinuria or rise in serum proteins had occurred. In nineteen children, this regimen produced a complete remission in 11, a complete remission except for slight residual proteinuria in 3 and a partial remission in 2. Three children failed to respond to steroid therapy and have since died. In thirteen adults, complete remission occurred in 4, complete remission except for residual proteinuria in 3, partial remission in 4, and two patients have failed to respond to steroid therapy, but are non-edematous.

In the past year, we have studied the effect of administration of chloroquine, an anti-malarial which often produces remissions in lupus erythematous, a disease which also responds to steroid administration. Seven patients have received 0.25 to 0.75 gm. of chloroquine phosphate daily for two to four weeks. Although a remission occurred in one patient and a partial remission in another coincident with chloroquine administration, in five other patients, essentially no change occurred. Subsequently, four of these

patients responded well to steroid therapy. Results to date therefore do not indicate any usefulness for chloroquine in the treatment of the nephrotic syndrome.

Current Research: Current studies include completion of the chloroquine assessment, and accumulation of more data on the effects of administration of high doses of steroids to patients who do not respond to the usual regimen of 40 mg. prednisone for three weeks to one month.

Part B. included Yes X

Part B. Honors, Awards and Publications

Publications:

1. Goodman, Howard and Baxter, James. Adrenocorticotrophic Hormone (ACTH) and Corticoid Treatment of the Nephrotic Syndrome: Metabolism (In Press).

2. Goodman, Howard and Baxter, James. The Nephrotic Syndrome: Clinical Observations on Therapy with Prednisone and Other Steroids. J.A.M.A. Vol. 165, 1799-1808, 1957.

Form No. ORP-2
Oct. 1957

Serial No. NHI-63 (c)

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar year 1957

Part A.

Project Title: Relationship between Albuminuria and Serum Albumin Concentration and between Serum Albumin Concentration and the Occurrence of Diureses in Nephrosis

Principal Investigators: James H. Baxter and Howard Goodman

Other Investigator: Patricia Duffy

Cooperating unit: None

Man Years (calendar year 1957) Patient days: 100
Total: 0.5
Professional: 0.4
Other: 0.1

Project Description:

Objectives a) To examine quantitatively the relationship between albuminuria and serum albumin concentration in nephrotic patients during periods of steady state with respect to these factors. b) To correlate the occurrence of diureses in patients with nephrosis with serum albumin levels. Also to study the mechanisms of diureses produced by steroid therapy.

Methods Employed: a) Albumin in the urine per day and serum albumin concentration have been determined during periods in which these values were steady for from one to several weeks. This was done during periods of active nephrosis and also during remissions of the disease. Albuminuria was expressed per sq. m. of body surface in order to compare patients of different body sizes. b) The serum albumin concentrations at the beginning and end of diureses induced by sodium restriction, steroid therapy, albumin infusions and spontaneous remissions have been determined.

Progress: a) It has been noted in 33 nephrotic patients that there was in general an inverse relationship between albuminuria per sq. m. per day and the concentration of serum albumin, with a considerable spread from one case to another at the higher levels of albuminuria. The maximum level of albuminurai observed in the steady state was 7.1 gm/sq. m/day, a rate slightly less than the estimated normal rate of albumin production. Unless the quantity of albumin degraded per day was increased above normal despite the great reduction in body

pool of albumin, the quantity of albumin produced per day in the present nephrotic patients was never greater than double the normal rate and usually was considerably less than this. Different levels of albuminuria (per sq. m./day) in patients with similar levels of serum albumin suggested that albumin production or fractional rate of degradation was abnormal in some of the patients.

b) Fifty-nine diureses of at least 5% of body weight were observed in 27 nephrotic patients. More than half had an incomplete diuresis, without any change in serum albumin, when hospitalized and placed on a low sodium diet. Sixteen patients later had diureses with complete loss of edema associated with increases in serum albumin concentration. Most of these occurred during steroid therapy. The serum albumin levels averaged 2.1 gm./100 ml. at the end of diureses. Diureses occurred in 6 patients with serum albumin concentrations averaging 0.9 gm./100 ml. upon discontinuing an ineffective course of steroid therapy.

The diureses associated with steroid therapy were of 2 types: 1) those occurring during steroid therapy associated with an increase in serum albumin concentration and probably resulting largely from the rise in serum albumin, and 2) those which follow termination of an ineffective course of steroid therapy, not associated with an increase in serum albumin level.

Direction of Current Research: These projects have been largely completed, though it is possible that further studies of albumin metabolism in nephrotic patients will be undertaken later using labeled albumin.

Part B. included No X

Form No. ORP-2
Oct. 1957

Serial No. NHI-64

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on antibodies to Tissue Antigens in Clinical and Experimental Nephritis

Principal Investigators: Howard Goodman and James Baxter

Other Investigators: Robert Bowser (Technician)

Cooperating units: None

Man Years: (calendar year 1957) Patient days: None
Total: 1.5
Professional: 0.6
Other: 0.9

Project Description: Immunization of rabbits with suspensions of human kidney tissue obtained at autopsy has produced antisera with high titers of antibodies to human kidney antigens. After absorption of such antisera with human serum and red blood cells (and even some other human tissue suspensions), high titers of antibodies against human kidney antigens are detectable by complement fixation and tannic acid hemagglutination (T.A.H.) tests. These rabbit antisera were utilized to show that the complement fixation and T.A.H. tests would give a positive reaction in our hands when we tested these known anti-kidney sera against extracts of human kidney. We then proceeded to test sera from 30 patients, utilizing these techniques, for the presence of "antibodies" against human kidney antigens. the patients included 2 with acute nephritis, 7 with nephrotic syndrome unresponsive to steroid therapy (of whom 3 have died), and six patients with nephrotic syndrome who have responded to steroid therapy, as well as other sera. No evidence for "anti-kidney antibodies" was found in any of these sera.

One serum from a patient with systemic lupus erythematosus had low titers of such "antibodies". When the serum was tested by the T.A.H. test against a nucleoprotein extract of human liver or kidney, high titers of "antibody" were found and changes in titer correlated well with the patient's clinical course. Several other patients with systemic lupus erythematosus also have a positive test in high

titer, although two patients with L. E. do not. Over 30 sera of patients with miscellaneous diseases have been negative in this test system. Preliminary results with elution of sera after starch block electrophoresis show that the "antibody" in the patient's serum is a gamma globulin, and is stable at 56° and survives freezing and thawing. The extract of liver or kidney nuclei with which the patients sera react contains nucleoprotein by spectrophotometric determination and its activity is destroyed by desoxyribonuclease and by trypsin.

Current Research: Further information about the positive test found in the serum of three patients with lupus erythematous will be sought in three ways.

a) More sera from patients with systemic lupus erythematous as well as other diseases will be screened to see which patients have a positive test.

b) Further characterization by electrophoresis (with Dr. Fahey in NCI) and ultracentrifugation (with Dr. Mihalyi) of the gamma globulin in the serum responsible for the positive test is planned.

c) Purification and characterization studies of the soluble substance extracted from liver and kidney nuclei will continue.

Part B. included Yes X

Part B. Honors, Awards and Publications

Publications:

1. Goodman, Howard and Baxter, James. The Search for Anti-Kidney antibodies in Patients. Proceedings of the Ninth Annual Conference on the Nephrotic Syndrome. (In Press).

Form No. ORP-2
Oct. 1957

Serial No. NHI-65

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the synthesis and degradation of proteins (Began August, 1956 - will end probably September, 1958).

Principal Investigators: Martha Vaughan and Daniel Steinberg

Other Investigators: Jane Logan and John Gorry (Technicians)

Cooperating units: None

Man Years: (calendar year 1957)
Total: 1.7
Professional: .7
Other: 1.

Patient days: None

Project Description:

General Purpose: To explore the mechanisms by which amino acids are assembled to form specific proteins and the possible role of intermediate compounds in this process.

Progress: A method for tritiation of organic compounds has been described by Wilzbach which makes it possible to prepare with relative ease a wide variety of tritium labeled compounds of high specific radioactivity. We have devised a method for preparation of protein or amino acid samples for tritium assay by the liquid scintillation counting technique. It was found that samples of these substances can be dissolved in a methanolic solution of a quaternary amine hydroxide. The resulting amine complex is soluble in the organic solvents required for solution of the phosphors employed in liquid scintillation counting. This method has been explored in some detail and has made it possible to carry out various studies with tritiated proteins and amino acids.

We have previously reported that the Wilzbach technique of tritiation is applicable to proteins. Tritiated lysozyme was prepared and subjected to chromatography on an ion-exchange column. A single major protein peak corresponding to that seen with untreated lysozyme was obtained. The specific radioactivity was constant throughout the peak as was the specific enzyme activity. Determination of protein, enzyme activity; and radioactivity before and after chromatography indicated the presence in the tritiated lysozyme of a small amount of enzymatically inactive protein of high specific radioactivity which remained bound to the resin after elution of the active lysozyme.

Similarly, ion-exchange column chromatography of tritiated ribonuclease yielded a protein fraction of high radioactivity without enzyme activity. However, this was incompletely resolved from the enzymatically active fraction. Thus, in the case of lysozyme and ribonuclease, although there is some degradation of protein during tritiation, a good yield of active enzyme can be obtained. Of course it is impossible to be certain that the tritiated molecules (which comprise only a small fraction of the whole) are still enzymatically active and unaltered structurally in a manner not detectable by the methods of purification used.

Direction of Research: Studies are in progress to determine the distribution of tritium in the amino acids of lysozyme labeled by this technique and to further characterize the labeled proteins.

The methods described above are now being employed in studies of protein biosynthesis and degradation (see Dr. Steinberg's report).

Part B. included Yes X

Part B. Honors, Awards and Publications

Publications:

1. Vaughan, M., Steinberg, D., Logan, J. Liquid Scintillation Counting of C^{14}-and H^3-Labeled Amino Acids and Proteins, Science 126, No. 3271, 446-47, 1957.

2. Steinberg, D., Vaughan, M., Anfinsen, C. B., Gorry, J. Preparation of Tritiated Proteins by the Wilzbach Method, Science 126, No. 3271, 447-48, 1957.

Form No. ORP-2
Oct. 1957

Serial No. NHI-66

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: To prepare and study physiological properties of disulfide albumin dimer.

Principal Investigator: Alan F. Hofmann (under supervision of Dr. Mihalyi.

Cooperating units: None

Man Years: (calendar year 1957)
Total: .5
Professional: .5
Other: 0

Patient days: None

Project Description:

Methods Employed: The mercurial albumin dimer was prepared by addition of mercuric chloride to buffered albumin solutions with crystallization from alcohol-water mixtures. The disulfide dimer is to be prepared by oxidation of the sulfur-mercury-sulfer bond with iodine at low temperatures. Sulfhydryl titration was performed using a mono-functional organic mercurial.

Progress: The mercurial dimer has been prepared with bovine serum albumin, but crystallization has not as yet been obtained. Studies to test the efficacy of mercurial dimer-monomer separation with a cellulose cationic exchange column are in progress.

Direction of Research: Human albumin, fraction V, is to be used for subsequent preparations of mercurial dimer, with crystallization from alcohol water mixtures planned. The disulfide dimer is to be tested for toxicity, in vivo stability, and stability in the presence of various biological reducing substances. Glomerular permeability is to be determined using the nephrotic rat for bio-assay, and the possibility of using the disulfide dimer as a means of restoring albumin levels in the hypoalbuminemic animal is to be evaluated.

Significance to Heart Research: Studies of plasma proteins are vital for comprehension of diseases where there are abnormal losses of protein, as in nephrosis. The mercury studies involve reactions with the sulfhydryl groups of proteins, these being of considerable significance in the activity of many hormones and enzymes.

Part B. included No X

Form No. ORP-2
October 1957

Serial No. NHI-67

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies of the effects of dietary fats on serum fatty acids in patients and animals.

Principal Investigator: Joel Avigan in collaboration with Donald Fredrickson

Other Investigator: Mary Ann Hurley (Technician)

Cooperating units: None

Man Years: (calendar year 1957) Patient days: None
Total: .7
Professional: .2
Other: .5

Project Description:

Progress: Three patients were kept on a low fat diet followed by a liquid formula diet containing either soybean, or safflower oil for 5-8 days. The low fat diet was reinstituted for 7-18 days, followed by a second formula containing coconut oil as the sole fat source for a similar period. Lipides from daily blood samples were extracted and fractionated chromatographically on a silicic acid column. The I Nos. of the fatty acids in the various fractions were subsequently determined. Rabbits were fed with diets containing 5% of fatty acid and their sera examined similarly after the termination of the experiment. Other animals kept on a standard diet were fed trace amounts of C^{14}-labeled fatty acids, 16 hrs. later their sera were drawn, fractionated and the specific activities of the fatty acids determined. Under all dietary conditions, both in patients and rabbits, the fatty acids of cholesterol esters were more unsaturated than those bound in phospholipides. Dietary fats affected appreciably the I Nos. of cholesterol esters and triglycerides, but only to a small extent the composition of phospholipides. Experiments with tracers clearly showed that the less saturated the ingested fatty acid, the greater the proportion found was esterified with cholesterol as compared to the amounts bound in riglycerides and phospholipides. The mechanism of the selective incorporation of different fatty acids into lipide moieties is being further investigated. The use of in vitro systems is contemplated for this purpose.

Part B. included No X

Form No. ORP-2
Oct. 1957

Serial No. NHI-68

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the chemical and physical properties of isolated serum lipoproteins.

Principal Investigator: Joel Avigan in collaboration with Christian B. Anfinsen

Cooperating units: None

Man Years: (calendar year 1957)
Total: .2
Professional: .2
Other: 0

Patient days: None

Project Description:

Progress: In order to reveal certain properties related to the primary structure of the protein moieties of the various lipoprotein classes the materials were subjected to partial enzymatic hydrolysis and subsequent high voltage paper electrophoresis of the products. Since the same proteins under identical conditions break down enzymatically into identical peptides, any difference in the products of controlled proteolysis of two classes of lipoproteins may be taken as an indication of a difference in the composition of the two substrates.

The proteins of serum lipoproteins Sf 3-8, and high density lipoproteins and also serum albumin were digested with subtilisin or with chymotrypsin. With both enzymes the digestion products of each of the three substrates were different. These results in conjunction with the previously published ones on the different N-terminal groups of the protein moieties of various serum lipoprotein classes emphasize the differences in the structure of these substances.

Careful determinations of -SH groups of both low and high density lipoproteins made by several methods gave negative results. It can be concluded that the extreme lability of lipoprotein solutions in the presence of air cannot be attributed to the oxidizability of -SH groups.

Current Research: Investigations of the chemical properties of the lipoprotein complex should be continued.

Part B. included: Yes X

Part B. Honors, Awards, and Publications

Publications:

1. Avigan, H. Modification of Human Serum Lipoprotein Fractions by Lipide Extraction. J. Biol. Chem. 226, 957-64, 1957.

Form No. ORP-2
Oct. 1957

Serial No. NHI-69

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the Mechanism of Protein Synthesis. (started August, 1956; will end probably September, 1958)

Principal Investigators: Daniel Steinberg, Martha Vaughan, Frederick Sherman and Alvin Markovitz

Other Investigators: John Gorry and Jane Logan (Technicians)

Cooperating units: None

Man Years: (calendard year 1957)
Total 2.8
Professional 1.9
Other .9

Patient days: None

Project Description:

General Purpose of Research: To establish the importance of the dynamic state of protein metabolism at the cellular level and to explore the possible involvement of intermediate compounds in protein synthesis.

Progress During Past Year: It has been shown that amino acid analogues can be incorporated into protein in vitro. This has been shown not only for the average mixed tissue proteins, as in most previous studies, but also for well characterized crystalline proteins. In particular, the incorporation of p-fluoro-phenylalanine into ovalbuman and into lysozyme by hen's oviduct has been established.

The incorporation of the analogues is energy dependent and proceeds at a somewhat decreasing rate for at least four hours. In this respect and also in regard to rate of incorporation the amino acid analogue behaves quite similarly to the normal amino acid.

It has been shown that when labeled amino acids are incubated with tissue slices or injected into the whole animal a rather large amount of the radioactivity found in the cell resides in the non-protein non-amino fraction. Even though the amount of radioactivity present in this fraction is large, the net amount of material is very small. In an attempt to obtain chemical identification of the bound amino acids in this fraction large scale preparations of lipid liver have been worked up and are being subjected to chromatographic and electrophoretic separations. A part of this work begun by Dr. Frederick Sherman while he was at NIH as a Visiting Scientist is being continued at Brown University by him.

Direction of Current Research: In order to determine the degree of selectivity in the protein synthetic mechanism a series of similar structural analogues will be examined. The important question is whether or not the cell synthesizes proteins as distinct entities or whether there is some latitude in the process. The use of analogues may help to answer this question.

Part B. included Yes X

Part B. Honors, Awards and Publications

Publications:

1. Markovitz, A. and Steinberg, D. A method for the determination of peptides in the presence of free amino acids. J. Biol. Chem. 228, 285-1957.

2. Steinberg, D., and Mihalyi, E. The Chemistry of Proteins. Annual Review of Biochemistry 26, 373-418, Palo Alto, Calif., Annual Reviews, Inc., 1957.

3. Rothberg, S. and Steinberg, D. Studies on the Mechanism of Enzymatic Decarboxylation. J. Amer. Chem. Soc. 79, 3274-78, 1957.

4. Steinberg, D. and Udenfriend, S. The Measurement of Radioisotopes. Methods in Enzymology, Vol. 4. Academic Press, New York, 1957.

Form No. ORP-2
Oct. 1957

Serial No. NHI-70

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Pathogenesis of atherosclerosis.

Principal Investigator: J. H. Bragdon. (Projects of Drs. Fredrickson and Gordon resemble, complement, and parallel this project).

Other Investigators: Carlos Schultz (technician) (Carl Lauter and Ed Mougey, technicians, although under my supervision, spent 50% of their time running a general service laboratory in lipid chemistry).

Cooperating units: None

Man Years (calendar year 1957):
Total: 3
Professional: 1
Other: 2

Patient Days (calendar year 1957):
None

Project Description:

Objectives: To study the pathogenesis of atherosclerosis through normal and abnormal lipid metabolism.

Methods: Serum lipoproteins are fractionated into several classes in the preparative ultracentrifuge. The fractions are quantitatively recovered and chemically analyzed for protein and lipid composition. The metabolism of the several classes are studied in animals by labelling with radioactive isotopes.

Progress During Past Year: The organ and tissue distribution of chylomicrons, labelled in the triglyceride with palmitic-acid-1-C^{14}, has been compared with that of the acid bound to albumin as UFA. In animals in positive caloric balance chylomicron counts are found primarily in the fat depots. UFA counts are found primarily in the striated muscle. In animals in negative caloric balance many chylomicron counts are found in heart muscle. This is not true of UFA counts. These findings indicate that both cardiac muscle and depot fat remove chylomicrons intact. These are also the sites at which the enzyme lipoprotein lipase is most abundant. It has been demonstrated in this laboratory that chyle chylomicrons in man, dog, and rat contain about 0.4% protein. Dogs and rats with cannulae in the thoracic duct have been fed labelled amino acids. The specific activity of the protein in the newly formed chylomicrons is highest in the first hour after feeding and declines exponentially. This suggests but

does not prove, that chylomicron protein is synthesized de novo in the gut wall. It was hoped that swine might prove a suitable laboratory animal for the study of atherosclerosis. They are ominivores and lesions resembling human atheromata have been reported in the aortae of slaughterhouse specimens. Accordingly, in cooperation with the Department of Agriculture Research Station at Beltsville, old boars were placed on diets which contained 40% of the calories either as corn oil or as butter. There was a significant increase in the serum cholesterol levels of the butter fed animals over their own control levels and over the levels in the corn-oil fed group. Fractionation of the lipoproteins indicated that the increase was limited to the alpha lipo-proteins. Atheromatous lesions were found in 50% of all animals, with no dietary correlations. It was concluded that swine are not a suitable species for the study of atherosclerosis.

Direction of Current Research: It is intended to seek further evidence as to the origin of chylomicron protein. The injection of protein-labelled chylo-microns should give additional evidence as to their eventual fate.

Part B included: Yes X

Part B. Honors, Awards, and Publications

Publications:

1. Bragdon, J. H., Havel, R. J., and Gordon, R. S., Jr. Effects of Carbohydrate Feeding on Serum Lipids and Lipoproteins in the Rat. Am. J. Physiol. 189, 63-67, 1957.

2. Bragdon, J. H., Zeller, J. H., and Stevenson, J. W. Swine and Experimental Atherosclerosis. Proc. Soc. Exper. Biol. & Med. 95, 282-284, 1957.

3. Bragdon, J. H. Lipoprotein Lipase. In a monograph edited by F. Homburger (In Press).

4. Bragdon, J. H. Current Trends in Atherosclerosis Research. AMA Archives of Industrial Health. (In Press).

5. 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. Inves. (In Press).

6. Bragdon, J. H. $C^{14}O_2$ Excretion after the Intravenous Administration of Labelled Chylomicrons in the Rat. Archives of Biochemistry & Biophysics. (In Press).

Honors and Awards relating to this project: None

Form No. ORP-2
Oct. 1957

Serial No. NHI-71(c)

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title:

1. Study of the mechanisms of fat transport and metabolism, utilizing tissues, animals, and man.

2. Studies of compounds and diets designed to lower plasma cholesterol.

3. Studies of patients with abnormal blood lipids.

Principal Investigator: Donald S. Fredrickson

Other Investigators: Duncan L. McCollester and Katsuto Ono (Technician).

Cooperating units: None

Man Years (calendar year 1957):
Total: 2.25
Professional: 1.35
Other: .9

Patient Days: 650

Project Description:

Methods: Radicactive fats or lipoproteins, especially those of very low density obtained from donor lymph, are injected into recipients or incubated in vitro to determine the manner of their metabolism. These studies include collaborative studies of labeled unesterified fatty acid (UFA) metabolism in man (with Dr. Gordon) and of further chemical characterization and purification of lipoproteins (with Dr. Redbell). Search for chemical inhibitors of cholesterol synthesis (with Dr. Steinberg) consists in screening likely compounds, toxicity tests, and trial in humans, if indicated. One such compound, Δ^4-cholestenone, has required investigation of its effects on production of adrenocortical hormones. Patients with abnormal blood lipids are admitted or seen in a weekly clinic, providing clinical material for trial of diets, drugs, tracer studies, and as sources of human lipoproteins.

Major Findings: Considerable understanding of the kinetics of unesterified fatty acid metabolism has been developed through the tracer studies, providing significant information on rates of transfer from plasma, magnitude of distribution, oxidation, and turnover. Metabolism has been shown to be generally similar for three acids, palmitic, oleic, and linoleic. Kinetics have been found similar in normals, hyperlipemics, diabetics, and one patient with the nephrotic syndrome.

The evidence of the importance of the metabolism of plasma UFA has been supplemented by recent studies of labeled UFA turnover in severe diabetic acidosis. The specific activity of urinary volatile ketones has been shown to quickly reach that expected if the blood ketones arise from the UFA "pool" defined by the tracer studies.

A method for the measurement of $C^{14}O_2$ in large samples of expired air has been developed to make tracer studies in humans at low levels of radioactivity possible. It is now possible to make over thirty analyses per day of specific activity in a human expiring less than 1×10^{-6} mc/minute $C^{14}O_2$.

The metabolism of chylomicron triglyceride fatty acids (TGFA) has been further clarified in that: (1) TGFA is found to be readily oxidized, and without the requirement of retransport in plasma as UFA; (2) Protamine experiments indicate that 'clearing' per se, probably does not depend upon an intact hydrolytic mechanism; (3) a dog burning almost exclusively carbohydrate or fat oxidizes less TGFA, but the pattern of chylomicron metabolism is not qualitatively altered; (4) arteriovenous differences across the heart have been measured by Dr. McCollester, using direct coronary sinus cannulation, with the finding of very low extraction, indicating that the heart prefers to metabolize UFA supplied from the blood, even when high levels of triglyceride are available.

The technique of adrenal vein cannulation has been used to demonstrate that chronic cholestenone administration may produce one of the most effective inhibitions of adrenocortical secretions reported in the rat. Hypertrophied glands in the drug-treated animals produce less than 3% of the normal corticosterone output per unit weight of gland.

Preliminary study of the metabolism of chylomicra tagged in the protein moiety (in collaboration with Dr. Bragdon) indicate that much of the protein of these lipoproteins may disappear at a rate different from that of the triglyceride.

Many dog and human lymph and plasma lipoprotein fractions have been prepared for characterization of the protein moiety by Dr. Rodbell. Such studies have already indicated that the D 1.019-1.063 lipoproteins are not related to the lipoproteins of D $\leq$ 1.006, including the chylomicra. Cellulose columns are being tried for further lipoprotein purification.

Dr. McCollester has found that a 30 gram fat diet, practical for outpatients, is as effective as large doses of safflower oil in maintaining a lower plasma cholesterol level in a carefully controlled group of hypercholesterolemics.

Proposed course of project: Major aims remain the same. Among the studies planned, the following specific ones are either in progress or contemplated.

(1) Expansion of the collaborative labeled UFA studies to include further study of diabetic acidosis, and the effects of certain parameters, such as exercise, on turnover rate.

(2) Collaborative studies of lipoprotein purification and characterization will include examination of any lipoprotein protein changes which may occur on transfer from lymph to plasma, chemical study of endogenous very low density lipoproteins, study of the metabolism of the labeled protein moiety of precisely defined very low density fractions. The complete end group pattern in the dog, and one other species will be compared with those in man to examine the premise that the "beta" lipoproteins in man may have arisen almost uniquely in this species for specific metabolic reasons.

(3) Triglyceride labeling in human chylomicra and very low density lipoproteins is being undertaken to permit studies comparable to those completed in the dog.

(4) Chylomicron TGFA metabolism is now being studied in the dog in terms of 1) rates of production and deposition of di- and monoglycerides, the behavior of C^{14} glycerol-labeled chylomicra, and A-V differences across certain tissues other than the heart.

(5) The adrenocortical inhibition produced by cholestenone in rats is being tested in other species to compare the effect of this compound on the output of 17-hydroxycorticosterone and aldosterone as well as corticosterone. The mechanism of this inhibition will be further investigated by testing rates of steroid synthesis from precursors, including acetate and cholesterol.

(6) The effect of the short chain fatty acids in milk in producing hypercholesterolemia in humans will be studied using formula diets as the beginning of a collaborative effort with the U. of Md. Dairy Department.

Significance to Heart Research: Understanding of the mechanisms of transport and intracellular metabolism of fats is essential to clarification of the role of lipids in atherosclerosis. The discovery of compounds affecting lipid synthesis and turnover and comparison of their *in vitro* and clinical effects may lead to effective therapy of hyperlipemic states, known to be associated with increased incidence of atherosclerosis. The broad application of current trends in diet therapy for hypercholesterolemia has wide practical importance.

Part B included: Yes X

Part B. Honors, Awards, and Publications

Publications:

1. Fredrickson, D. S., and Steinberg, D. Failure of alpha-phenylbutyrate and beta-phenylvalerate in treatment of hypercholesterolemia. Circulation 15, 391, 1957.

2. Fredrickson, D. S. Some biochemical aspects of lipid and lipoprotein metabolism. J.A.M.A. 164, 1895-99, 1957.

3. Horning, M. G., Fredrickson, D. S., and Anfinsen, C. B. Studies on the enzymatic degradation of the cholesterol side chain. II. Requirements of the mitochondrial system. Arch. Biochem. Biophysics 71, 266-73, 1957.

4. Fredrickson, D. S., McCollester, D. L., Havel, R. J., Ono, K. Early steps in transport and metabolism of exogenous triglyceride and cholesterol. Symposium: Chemistry of lipids as related to atherosclerosis, Cleveland, May, 1957 (In Press).

5. Fredrickson, D. S. Lipide transport mechanisms and their inter-relationships, Symposium: The Metabolic Conference, Palm Springs, February, 1957 (In Press).

6. 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. (In Press).

7. Fredrickson, D. S. and Gordon, R. S., Jr. The Metabolism of unesterified fatty acids in Man. Symposium: Biochemical Problems of Lipids, Oxford, 1957. (In Press).

8. Fredrickson, D. S. Atherosclerosis. for text book. Metabolic diseases of the Cardiovascular System. Glasgow (In Press).

Honors and Awards: None

Form No. ORP-2
Oct. 1957

Serial No. NHI-72

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Design and Construction of apparatus for Vapor Phase Chromatography.

Principal Investigators: Dr. Arthur Karmen and Dr. Robert Bowman

Other Investigators: Harold Tritch and David Shear.

Cooperating units: This project was supported by funds from and performed jointly with the Laboratory of Technical Development, NHI.

Man Years (calendar year 1957): Patient Days: None
Total: 1.02
Professional: .7
Other: .32

Project Description:

Progress: A series of vapor phase chromatography gas sensing devices were constructed, employing the principle of the glow discharge tube. It was possible to construct tubes of many different designs which were successful in differentiating minute changes in gas composition. The sensitivity of these devices was comparable with the most sensitive devices recently reported in the literature.

Current Research: Is directed toward the improvement of the stability of the system and the demonstration of its applicability to high temperature chromatography of fatty acid esters.

Part B. Included: No X

Form No. ORP-2
Oct. 1957

Serial No. NHI-73

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Design of a method for the measurement of trypsin in blood.

Principal Investigator: Arthur Karmen

Other Investigator: B. J. Haverback

Cooperating units: None.

Man Years (calendar year 1957): Patient days: None
Total: .43
Professional - .43
Other 0

Project Description:

Progress: A specific synthetic substrate for trypsin was made experimentally applicable to the measurement of trypsin in the presence of serum and plasma in vitro. In vitro studies of the reaction of added crystalline trypsin with plasma trypsin inhibitor were performed. It was found that competition between plasma trypsin inhibitor and added synthetic substrate for combination with added trypsin was observable. The competition, however, was not of the usual "competitive inhibition" type. In the presence of excess quantities of plasma trypsin inhibitor, a constant fraction of the trypsin added was measurable, and this finding indicated a method of measurement of trypsin as might be found in plasma or serum. Crystalline trypsin was given intravenously to dogs. The volume in which this trypsin was distributed was calculated to be approximately equal to the plasma volume.

Measurement of endogenous trypsin in plasma was attempted. No trypsin was found in ten patients sera. No trypsin was measurable in the sera of three dogs prior to the administration of crystalline trypsin.

In vitro incubation of an extract of acetone powder, of duodenum, a source of enterokinase, with plasma resulted in the appearance of active trypsin in the mixture, indicating the possibility of the existence of the enzyme precursor trypsinogen in plasma.

Current research: To be directed along the lines of preparing a more purified enterokinase preparation and attempting, by differential activity toward several synthetic substrates, to differentiate the enzyme activity noted after incubation of plasma with duodenal extract from the plasmin enzyme system.

Significance to Heart Research: Administration of crystalline trypsin has been proposed as a treatment for thrombophlebitis and other conditions. No method for determining blood levels of trypsin following its administration has heretofore been available.

Part B. included No X

Form No. ORP-2
Oct. 1957

Serial No. NHI-74(c)

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Clinical Studies on Plasma Proteins.

Principal Investigator: Robert S. Gordon, Jr.

Other Investigators: John Davidson (Exper. Therapeutics Branch)

Cooperating units: Dr. Jesse Steinfeld, NCI, has collaborated on this research.

Man Years (calendar 1957): Patient Days: 350
Total .85
Professional: .85
Other: 0

Project Description:

Progress: The abnormality of protein metabolism in regional enteritis and ulcerative colitis has been related to loss of albumin and other plasma proteins from the circulating plasma into the lumen of gastro-intestinal tract. Studies with labeled albumin and labeled polyvinyl pyrrolidone (PVP) indicate that this abnormal route of metabolism may cause the breakdown of considerably more plasma protein than is normally degraded, resulting in hypoproteinemia.

The use of electrophoretic analysis to determine the rate of disappearance of passively infused gamma globulin in agammaglobulinemic patients has continued, extending and confirming earlier observations.

Current Research: The electrophoretic apparatus remains available and will be used to assist any investigators who require accurate plasma protein determinations. The studies of plasma protein catabolism in gastro-intestinal diseases will be pursued with the objective of developing isotopic PVP into a generally applicable diagnostic procedure for the detection of enteritis and colitis.

Part B. included Yes X

Part B. Honors, Awards, and Publications

Publications:

1. Martin, C. M., Gordon, R. S., Felts, W. R. and McCullough, N. B. Studies on Gamma Globulin. I. Distribution and Metabolism of Antibodies and Gamma Globulin in Hypogammaglobulinemic Patients. J. Lab. & Clin. Med. 49, 607-616, 1957.

Form No. ORP-2
Oct. 1957

Serial No. NHI-75

1. Laboratory of Cellular Physiology & Metabolism
2. Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Interactions of fatty acids with human serum albumin

Principal Investigator: DeWitt S. Goodman

Cooperating units: None

Man Years (calendar 1957):
Total: 1
Professional: 1
Other: 0

Patient Days: None

Project Description: The interaction between human serum albumin and long-chain fatty acids.

Methods: Varying quantities of isotopically labelled fatty acids are equilibrated between an aqueous and a nonaqueous phase in the presence and in the absence of human serum albumin. By means of such measurements over a wide range of fatty acid concentrations the characteristics of the interaction of the fatty acid with the protein can be defined.

Progress: The interactions of human serum albumin with a number of long-chain fatty acid anions have been completely defined. The fatty acids studied have included lauric, myristic, palmitic, stearic, oleic, and linoleic acids. For each case the number of binding sites and the appropriate association constants have been determined.

During the course of this project it became necessary to study the dimerization of fatty acids in heptane and the partition of fatty acids between heptane and phosphate buffer. Dimerization (association) constants have been determined for octanoic, decanoic, laurice, and myristic acids. Anomalous behavior has been found for the other fatty acids listed above. This project, which began in July, 1956, was completed in October, 1957.

Current Research: The interaction of long-chain fatty acids with human red cells is being investigated as a first step towards the investigation of cellular receptor sites for unesterified fatty acids.

Part B. included Yes X

Part B. Honors, Awards, and Publications

Publications:

1. Goodman, DeW. S. Preparation of Human Serum Albumin Free of Long Chain Fatty Acids. Science 125, 1296-97, 1957.

Honors and Awards: 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 1958

Total:	$215,858
Direct:	$155,846
Reimbursements:	60,012

Form No. ORP-2
Oct. 1957

Serial No. MHI-75(c)
1. Laboratory of Cellular Physiology
2. Section on Enzymes
3. Bethesda, Maryland.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Requirements for Nucleotidase Action

Principal Investigators: Emery C. Herman, Jr.
Other Investigators: Barbara E. Wright

Cooperating Units:

Man Years (calendar year 1957): Patient Days (calendar year 1957):
Total: ½ 180
Professional: ½
Other:

Project Description:

1. General Purpose of Research: To study the requirements for nucleotidase action.

2. Progress During the Past Six Months: An enzyme capable of catalyzing the cleavage of the mono-, di-, and tri-phosphoric acid esters of the purine and pyrimidine nucleosides has been obtained in soluble form from extracts of the anaerobe, clostridium sticklandii and has been purified to a seven-fold extent. It has been shown that this nucleotidase action is entirely dependent upon reducing agents and in more purified preparations upon metallic (rather than organic) reductants. Evidence points to ferrous iron as the principal activator. The characteristics of the enzyme and certain parameters of the reaction have been studied and form the basis of a descriptive report now ready for submission. It is felt that a single enzyme is responsible for the cleavage of all the nucleotide substrates.

3. Direction of Current Research: A further investigation into the mechanism of pteridine interplay in the conversion of pyruvate into acetate and CO_2 is planned. Previous work by Wright et al has described the evidence implicating simultaneous pteridine reduction in a clostridial pyruvate oxidase system. A major portion of the pyruvate is being metabolised by means of an arsenate-sensitive pathway (presumably the lipoic-acid dependent system), and an attempt will be made to separate this enzymic system from the pteridine dependent pathway.

4. Incidental Findings of Significance: None.

Part B included Yes X No

Serial No. NHI-76(c)

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, AWards, and Publications

Wright, B.E., Anderson, M.L., and Herman, B.C Jr.,
The Role of Polyglutamyl Pteridine Coenzymes in Serine Metabolism.
III The Enzymatic Formation of Dihydrofolic and Dihydroteropterin.
J. Biol. Chem. Jan 1958 (in press).

Form No. CRP-2
Oct. 1957

Serial No. NHI-77
1. Laboratory of Cellular Physiology
2. Section on Enzymes
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Synthesis, Isolation and Characterization of Compounds of Biological Interest.

Principal Investigators: H. Todd Miles

Other Investigators: K. Chaney

Cooperating Units:

Man Years (calendar year 1957): Patient Days (calendar year 1957):
Total: 1-1/8
Professional: 1.0
Other: Technical: 1/8

Project Description:

1. General Purpose of Research: 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.

2. Progress During the Past Ten Months: Further studies of infrared spectra in D_2O solution of new model compounds were used to draw conclusions about tautomeric structure of the naturally occurring pyrimidine nucleosides and nucleotides. In addition, the use of information from the infrared spectra of model compounds and from Cochrane's x-ray analysis of adenine sulfate permitted the tautomeric structure of adenosine and its phosphate esters to be assigned for neutral and acid solution.

The structure of a new bacterial metabolite of riboflavin, which was isolated by P. Z. Smyrniotis and E. R. Stadtman, was shown to be 3,4 dimethyl-6-carbony-α-pyrone by means of chemical degradation experiments. This is the first time an α-pyrone has been shown to be derived biologically from an aromatic compound and is, in fact, the first monocyclic α-pyrone to be isolated from a natural source.

3. Direction of Current Research: Work is in progress on the synthesis and chemistry of the newly isolated α-pyrone and some closely related compounds.

4. Incidental Findings: None.

Part B Included Yes X No

Form No. CRP-2
Oct. 1957

Serial No. NHI-77
1. Laboratory of Cellular Physiology
2. Section on Enzymes
3. Bethesda, Maryland.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B. Honors, Awards, and Publications

Miles, H. Todd, "Synthesis of Some Model Pyrimidine Nucleosides" J. Am. Chem. Soc. 79, 2565 (1957)

Miles, H. Todd, "Infrared Spectra and Tautomeric Structure of Nucleosides and Nucleotides, II", Biochim. Biophys. Acta, in press.

Form No. [illegible]
Oct. 1957

Serial No. NIH-7[illegible]
1. Laboratory of Cellular Physiology
2. Section on Enzymes
3. Bethesda, Maryland.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: I. Pterine Metabolism
II. Biochemistry of Differentiation in the slime molds.

Principal Investigator: Barbara E. Wright

Other Investigators: Emery C. Herman and Minnie L. Anderson

Cooperating Units:

Man Years (calendar year 1957): Patient Days (calendar year 1957
Total: 2-3/4
Professional: Project I - 3/4
Project II - 3/4
Other: Technical - Project I - 5/8
Technical - Project II - 5/8

Project Description:

1. General Purpose of Research: I. To investigate the role of Pterines in intermediary metabolism. II. To understand the enzymatic basis for differentiation in a system simple enough to allow a meaningful analysis.

2. Progress During the Past Six Months:

I. The unidentified pterine discussed in the last report turned out to be a dihydropterin. The unidentified cofactor was identified as CoASH. To make too long a story short:

Teropterin + CoASH + pyruvate ——— Teropterin H_2 + AcSCoA + CO_2.

The previously observed phosphate and serine requirements were for the purpose of regenerating CoASH (via phosphotransacetylase) and generating pyruvate, respectively. Phosphate is not required in the presence of substrate levels of CoASH, and pyruvate is a better electron donor than serine for dihydro pterine formation. The above reaction is of interest for pterine metabolism in that it represents a mechanism by which pterines are kept reduced, in order to function in one carbon transfer reactions. The reaction also places pterines in a new light with respect to their possible role in electron transport.

II. The life cycle of the slime mold can be summarized as follows: When the individual, vegetative myxamoebae are deprived of food, they can be plated on wet glass or washed agar - in the absence of any nutrients - and they will begin to aggregate in streams towards a central point in response to a diffusable, chemotactic substance formed by the amoebae themselves.

The aggregate (about 150,000 cells) forms a migrating slug, the rear end of which are the presumptive spore cells and the front end of which are the presumptive stalk cells in the final fruit. The apical tip of the slug starts synthesizing cellulose, and the rear presumptive spore cells, are pulled up, in part due to this synthesis, until the spore sac (composing 85% of the cells of the slug) sits on top of the visible cellulose stalk. This whole process of differentiation can occur within 12 hours.

It is possible to assay for the chemotactic substance, and it was felt that crude hormone preparations should be tested. The urine of a pregnant woman was active, as were a number of steroids tested thereafter. It was recently found that estrone SO$_4$ is active at 0.01γ/ml.

Enzymatic changes during differentiation were examined. Such changes should be referable only to differentiation, and not confused with phenomena of growth. Striking increases in the activity of a number of "constitutive" enzymes (eg. Zwischen ferment, isocitric dehydrogenase) were observed, as well as in the enzyme synthesizing uridine diphosphoglucose (U D P G pyrophosphorylase). The latter enzyme was investigated because it was recently reported that U D P G is the activated form of glucose for cellulose synthesis. Cellulose synthesis in vitro has been examined also, with preliminary positive results. Thus far, the nature of the enzymatic changes occurring, as well as the time at which they occur make sense with respect to the concurrent pattern of differentiation.

3. Direction of Current Research:

It is first necessary to obtain some more descriptive, quantitative data on the enzyme changes, and correlate them in time with the observed morphological changes. Two of the major problems seem to be considered are:

1. Are the increases in enzymatic activity due to protein synthesis, or activation?
2. How does the hormone act?

4. Incidental Findings of Significance:

None.

Part B included Yes X No

Serial No. NHI-78

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

1. B. E. Wright and M. L. Anderson, Folic Acid Reductase. J. Am. Chem. Soc. 79 2027 (1957)

2. B. E. Wright, M. L. Anderson and E. C. Herman, Jr. The Role of Polyglutamyl Pteridine Coenzymes in Serine III The Enzymatic formation of Dihydrofolic and Dihydroteropterin. J. Biol. Chem. Jan., (1958) in Press.

3. B. E. Wright and M. L. Anderson, Pterine Reductase. Biochim. et Biophys. Acta, in Press.

Honors:

Invited to present a symposium paper on Pterins at the IV International Biochemical Congress.

Form No. CRP-2
Oct. 1957.

Serial No. NHI-79
1. Laboratory of Cellular Physiology
2. Section on Enzymes
3. Bethesda, Maryland.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: A. Intermediary Metabolism of Amino Acids with particular emphasis on those reactions involving overall reductive deaminations.

B. Anaerobic Metabolism of C_1 Compounds and Methyl-Group Donor Compounds.

Principal Investigators: Thressa C. Stadtman
Hugh R. Hayward

Other Investigators: John Hardman (since June 1957)
Lydia Tiemann (January - June 1957)

Cooperating Units:

Man Years: (calendar Year 1957): Patient Days (calender year 1957):
Total: 2-3/8
Professional: 1½
Other: 1-1/8

Project Description:

1. General Purpose of Research: A. 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. B. To study the anaerobic metabolism of compounds such as choline, betaine, and mono and dimethyl derivatives of glycine and ethanolamine from the standpoint of (1) nature of active one-carbon fragments that enter into condensation reactions to form such compounds as acetate and (2) types of energy-yielding processes involved in cleavage of these C-N compounds.

2. Progress During the Past Year:

A. Organisms that ferment Δ-amino valeric acid and γ-amino butyric acid have been obtained by the enrichment culture technique. These fermentations of single amino acids are coupled oxido-reduction reactions wherein one mole of the substance undergoes oxidation while a second acts as electron acceptor and becomes reduced. Thus for Δ-amino valerate one obtains:

(1) $$\underset{\displaystyle NH_2}{CH_2}CH_2CH_2CH_2\,COOH \xrightarrow{2H_2O} CH_3CH_2COOH + CH_3COOH + NH_3 + 2H$$

(2) $$\underset{\displaystyle NH_2}{CH_2}\text{-}CH_2CH_2CH_2COOH + 2H \longrightarrow CH_3CH_2CH_2CH_2COOH + NH_3$$

Sum: 2 Δ-amino valerate ——— 1 propionate + 1 acetate + 1 valerate + $2NH_3$

Reaction (1), as determined by isotope studies, involves a β-oxidation mechanism. Reaction (2) is a reductive deamination analogous to glycine reduction to acetate. Glycine conversion to acetate has been found to be an energy-yielding process that results in the formation of ATP but the nature of the phosphorylated intermediate is as yet unknown. Preliminary experiments indicate that the Δ-amino valerate breakdown in extracts also involves a phosphorylation step and efforts are being made to gain further insight into the nature of reductive deamination processes by examining the metabolism of these related substrates with biological preparations that may be more satisfactory for this phase of the general problem.

Continued work on the glycine reductase of C. sticklandii has revealed that purification of the enzyme system to the point where very little acetate is formed yields a preparation that still shows high ATP-formation. Associated with this glycine and mercaptan-dependent phosphate esterification reaction is the conversion of glycine to a compound volatile from alkaline solution. Attempts are currently in progress to establish the nature of this product which may be closely related to an intermediate in the overall conversion of glycine to acetate.

B. A series of microorganisms which grow anaerobically on the mono, di- and tri-methyl derivatives of glycine and ethanolamine have been obtained by the enrichment culture technique. Carbon balances and isotope experiments carried out on growing cultures reveal the following:

Choline $(CH_3)_3 N^+ CH_2CH_2OH$ ——— $(CH_3)_3N$ + ½ ethanol + ½ acetate + H^+

$(CH_3)_2 N CH_2CH_2OH$ ——— /mole of base + ½ acetate and no alcohol.

$CH_3 NH CH_2CH_2OH$ ——— $CH_3 NH_2$ + acetate + alcohol
ca.½ ca-½

Betaine $(CH_3)_3N^+ CH_2COOH$ ——— /mole acetate + sarcosine or a derivative, + H^+ (no volatile base or alcohol formed)

The cleavage reactions, in general, appear to be reductive in nature and may involve new types of phosphorylation steps. The betaine organism looks particularly promising as biological material for study of C_1 condensations to yield acetate. Experiments currently in progress with ^{14} labelled betaine will establish the validity of this belief. The apparent requirement of several of these organisms for CO_2 for growth also points to very active C_1 metabolic systems.

3. Incidental Findings of Interest:

During attempts to phosphorylate SH^- compounds using p-nitro phenyl phosphate as phosphorylating agent, it was discovered that an enzyme preparation of C. sticklandii contains an alkaline phosphatase that will cleave this ester if an SH^- compound is added. Furthermore, upon aging, these preparations become more and more dependent upon the simultaneous addition

of menadione or similar 1,4-naphthequinones at catalytic levels. A compound more similar to Vitamin K, phthiocol, does not activate the phosphatase. The natural substrate of this enzyme is as yet unknown, but when glycine reduction is studied with these same enzyme preparations it is interesting to note that this process with its attendant phosphorylation is inhibited by the quinones that stimulate the phosphatase, and relatively unaffected by those that do not. One wonders if a phosphorylated intermediate involved in glycine reduction is hydrolyzed when menadione or other K analogues is added.

Another incidental finding was that crystalline commercial alcohol dehydrogenase contains a phosphatase that hydrolyzes p-nitro phenyl phosphate and also phosphoramidate but not other common esters. This appears not to have been previously reported. Both activities (phosphatase and dehydrogenase) are heat labile, are sensitive to SH-binding agents and behave similarly on various absorption gels. Experiments are being continued to see if this could be an example of a single protein with 2 different enzyme activities.

4. Direction of Current Research:

Studies at the enzyme level on the amino acid reductase systems to obtain information about

(a) Chemical intermediates involved in conversion of amino acids to fatty acids and nature of cyclic processes employed.

(b) Types of energy-rich intermediates synthesized that enable the organisms in question to satisfy their energy requirements for growth by carrying out these processes.

(c) Mechanism of ammonia release

The overall fermentation processes involved in the anaerobic utilization of N-methyl derivatives of ethanolamine and glycine are being mapped out by means of C, N and H balance experiments and also by utilization of isotopically labelled substrates. After completion of this phase of the problem the various microorganisms will be cultivated on a large scale, cell-free extracts will be prepared and (1) attempts will be made to learn something about the more detailed mechanism of the energy-yielding steps involved in the utilization of the various N-methyl compounds under investigation (2) The extracts will be screened as to their ability to activate various one-carbon compounds or one-carbon donor compounds and to synthesize more complex molecules from same.

Some collaborative experiments with Dr. D. Shemin of Columbia University will be carried out on the general problem of relationship of Vitamin B_{12} to C_1 metabolism - in particular methane formation. The methane bacteria, a group of organisms whose whole metabolism is geared to the transformation of CO_2 to CH_4, are unusually high in vitamin B_{12}. Dried cells of one of these reduce CO_2 to CH_4 with molecular H_2 and such preparations will be tested for ability to convert C^{14}-labelled B_{12} (prepared by Shemin) to $C^{14}H_4$ methane.

Part B included Yes X No.

Serial No. NHI-79

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

1. Stadtman, Thressa C. and Patricia Elliott "Studies on the Enzymic Reduction of Amino Acids II Purification and Properties of a D-proline Reductase and a proline Racemase from Clostridium Stickladdii " J.B.C. 228: 983 (1957)

2. Stadtman, Thressa C., Elliott, Patricia and Tiemann, Lydia, "Phosphate Esterification Coupled with Glycine Reduction" J.B.C. (in press).

Form No. ORP
Oct. 1957

Social Security No. [illegible]
1. Laboratory of Cellular Physiology
2. Section on Enzymes
3. Bethesda, Maryland.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The enzymatic synthesis of "energy-rich" compounds and their utilization in biosynthetic reactions.

Principal Investigators: Roy Vagelos
E. R. Stadtman

Other Investigators: Joan Earl, K. Chaney, B. Richards, R. Stroud.

Cooperating Units:

Man Years (calendar year 1957): Patient Days (calendar year 1957)
Total: 2-5/8
Professional: 1.0
Other: Technical 1-1/8
Patient Care ½

Project Description:

1. The enzymatic synthesis of 'energy-rich' compounds and their utilization in biosynthetic reactions.

2. Progress During the Past Six Months:

Propionate Metabolism: The enzyme that converts acryly-pantetheine (or acrylyl-CoA) to β-alanyl pantetheine has been further characterized. An enzyme has been found in a Pseudomonas Sp. and in pigeon heart that converts acrylyl-pantetheine to lactyl-pantetheine. The latter compound has been synthesized chemically (the CoA derivative) so that the fate of this compound can be studied. An assay has been worked out that should locate a lactyl-CoA dehydrogenase if there is one present in the microorganisms we have available.

Acrylyl pantetheine is also metabolized by extracts of cl. kluyveri the product here being β-hydroxypropionyl-pantetheine. The latter is further metabolized to a labile compound that has a U.V. absorption at 308 mμ. A heat stable cofactor, not yet identified, is associated with that reaction. Propionic, acrylic, and β-hydroxypropionic acids can be activated to their CoA derivatives by this organism - all leading to the production of the unidentified '308 compound'.

Metabolism of Heterocyclic Compounds:

In an effort to study the more intimate metabolism of certain drugs

and alkaloids, enrichment cultures were set up on the following substrates: morphine sulfate, amphetamine, ephedrine, atropine, theophylline, barbituric acid, lysergic acid, thyroxine, triiodothyronine. Organisms have been isolated that presumably have these compounds as their sole source of carbon, nitrogen and energy.

3. Direction of Current Research:

All the above problems will be continued. Immediate work will be on identification of the "308 compound" and searching for a lactyl-CoA dehydrogenase. When this work is complete, full attention will be given to study of the organisms that were isolated from drug enrichment cultures.

Part II Included Yes X No

Serial No. NHL-80

Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

1. Stadtman, E.R., Preparation and Assay of Acetyl Phosphate, Methods in Enzymology, 238, Vol. III, edited by Colowick, S.P. and Kaplan, N.O., Academic Press, Inc., Publishers, New York, 1957.

2. Stadtman, E.R., Preparation and Assay of Acyl Co-enzyme A and other Thiol Esters; Use of Hydroxylamine; Methods in Enzymology, 931, Vol. III, edited by Colowick, S.P. and Kaplan, N.O., Academic Press, Inc., Publishers, New York 1957.

3. Kornberg, A. and Stadtman, E.R., Preparation of Coenzyme A Method in Enzymology, 907, Vol. III, edited by Colowick, S.P. and Kaplan, N.O, Academic Press, Inc., Publishers, New York 1957.

Honors:

E. R. Stadtman - Received 1956 Washington Academy of Sciences Award for Scientific Achievment in the Biological Sciences (awarded Jan. 17, 1957).
Elected to membership in the Washington Academy of Sciences.
Received Travel Award from Federation of American Societies for Experimental Biology to attend Enzyme Symposium in Japan.
Elected to Executive Committee of the Division of Biological Chemistry of the American Chemical Society.

Form No. CRP-2
Oct. 1957

Serial No. NHI-61
1. Laboratory of Cellular Physiology
2. Section on Enzymes
3. Bethesda, Maryland.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Enzymatic Degradation of Cholesterol by Mammalian and Insect Enzyme Systems.
Metabolism of Isoprenoid Structures.

Principal Investigator: Marjorie G. Horning.

Other Investigators: Technical - K. Chaney.

Cooperating Units:

Man Years (calendar year 1957): Patient Days (calendar year 1957):
Total: 1-1/8
Professional: 1.0
Other: Technical - 1/8

Project Description:

1. General Purpose of Research:- Current concepts of atherosclerosis are generally based on the belief that atherosclerotic lesions are a consequence of faulty cholesterol metabolism, induced by a variety of causes, including distortions in normal fatty acid metabolism and in hormonal metabolic control systems. The key to cholesterol metabolism lies in the first few steps by which the side chain is oxidized, and the molecule started toward the bile acid end point. These reactions have been studied with mammalian and insect preparations.

2. Progress During the Past Six Months:-

a) Cholesterol was found to undergo a rapid metabolic change with an acetone powder of sawfly larvae (neodiprion pratti pratti). The initial reaction product has been isolated by chromatography on silicic acid columns. It is apparently a molecular compound containing at least two cholesterol derivatives. The formation of molecular compounds is common for sterols. This material shows strong carbonyl absorption in its infrared spectrum. It has been fractionated into two substances which now require identification. This work has been done with radioactive cholesterol.

Two additional fractions have also been studied. A more polar substance, containing some radioactivity, has been obtained in column separations. This may correspond to a later stage of metabolism. A less polar fraction has been isolated. This material was not radioactive and, therefore, was not directly derived from cholesterol. However, the yield of this material is proportional to the amount of substrate, and may, therefore, play some part in the metabolic process. This fraction appears to

contain fatty acids.

b) Exploratory work indicated that an acetone powder of sawfly larvae contained enzymes capable of metabolizing a number of isoprenoid structures. In addition to β-ionone (reported previously) it was found that Vitamin K and Vitamin E would undergo metabolic change. While these observations should be followed, there was not sufficient time for further investigation.

c) Studies were described in the previous report on the isolation of a cofactor that is required for the oxidation of the terminal carbon of the cholesterol side chain. This involves the cholesterol-bile acid conversion route. Cofactors are generally of considerable metabolic significance, and the identification of this material constitutes an important problem which should be pursued intensively.

This work was discontinued because it had reached the stage where routine technical assistance was essential for further progress. Support for the project was requested several times, but, in the face of a total lack of interest in the problem and lack of support, no further work was done, and the work to date was published.

This project bears directly on the atherosclerosis problem. While it, undoubtedly, will be picked up and pursued elsewhere, much time will be lost that might be used in arriving at further results.

d) It was brought to the author's attention that two varieties of the Magueyworm, a native of Mexico, were able to metabolize steroids. With the help of the U.S. Agricultural Attache and the Rockefellar unit in Mexico City, a supply of these larvae was collected and converted to an acetone powder. The cholesterol-metabolizing effects of this material are under study.

3. Direction of Current Research

This work on cholesterol metabolism will be carried out for the next six months under the direction of Dr. J. W. Cornforth and Dr. G. Popjak in London.

[illegible] Included Yes X No

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

1. Horning, M.G., Fredrickson, D., and Anfinson, C.B., Studies on the Enzymatic Degradation of the Cholesterol Side Chain, Archives of Biochem. Biophys. 71, 266 (1957).

2. Horning, M. G., Sterol Requirements of Insects and Portozoa "Cholesterol", Edited by R. Cook, Academic Press.

3. Horning, M. G., Degradation of Cholesterol by Acetone Powder of Sawfly Larvae, 131st Meeting, Am. Chem. Soc. April, 1957.

Form No. ORP-2
Oct. 1957

Serial No. NHI-82
1. Laboratory of Cellular Physiology
2. Section on Enzymes
3. Bethesda, Maryland.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Bacterial Degradation of Creatinine

Principal Investigator: Dr J. Szulmajster, Visiting Scientist

Cooperating Units:

Man Years (Calendar year 1957): Patient Days (calendar year 1957)
Total: 1.0
Professional: 1:0
Other:

Project Description:

Two anaerobic microorganisms (Bs and Bl) belonging to the genus of clostridium have been isolated from soil by means of enrichment culture technique, able to grow on creatinine.

Clostridium Bs degrades creatinine during growth with the formation of NH_3 and N-methyl-hydantoin in equimolar amounts. N-methyl-hydantoin which accumulates during growth has been isolated and crystallized in highly pure state.

In order to study the mechanism of this reaction, the enzyme has been isolated from cell-free extracts and purified 4300 fold using a 3 step purification procedure involving protamine, ammonium sulfate and Sober & Peterson's anion exchange resin (DEAE-SF). The properties and kinetics of the enzyme have been studied in detail.

Growth of Clostridium Ls shows an absolute requirement for creatinine. Sarcosine, 2 NH_3 and CO_2 are the end products of this creatinine fermentation.

The complete mechanism of these reactions is now being investigated.

Part F included Yes No X

Form No. ORP-2
Oct. 1957

Serial No. NHI-83
1. Laboratory of Cellular Physiology
2. Section on Enzymes
3. Bethesda, Maryland.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Riboflavin Degradation.

Principal Investigators: P. Z. Smyrniotis
Earl R. Stadtman

Other Investigators: Richard Stroud, Benjamin Richards, Albert Johnson, Kathrin Cheney.

Cooperating Units:

Man Years (calendar year 1957): Patient Days (calendar year 1957):
Total: 1-5/8
Professional: 1½
Other: Technical - 1/8

Project Description:

An aerobic microorganism has been isolated from soil by means of the enrichment culture technique which degrades riboflavin. Large scale aerobic oxidation of riboflavin by this microorganism has resulted in the accumulation of numerous degradation products in the culture medium. By means of chromatography on florisil columns, gram amounts of products have been obtained. The first product identified was 3,4-dimethyl-α-pyrone, and constitutes the first instance in which a mono-cyclic α-pyrone derivative has been found.

The nature of a second purified product, a precursor of the α-pyrone, is under investigation.

Part B included Yes No X

Form No. ORP-2
Oct. 1957

Serial No. LMI-8A
1. Laboratory of Cellular Physiology
2. Section on Enzymes
3. Bethesda, Maryland.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Enzymatic Mechanisms in Clostridium kluyverii.

Principal Investigator: Dr. S. C. Kinsky
(Public Health Service Postdoctoral Fellow)

Sponsor: Dr. E. R. Stadtman

Other Investigators: K. Chaney

Cooperating Units:

Man Years (calendar year 1957): Patient Days (calendar year 1957)
Total: 7/8
Professional: 3/4
Other: Technical - 1/8

Project Description:

1. General Purpose of Research: Research during the period March 1, 1957 - October 30, 1957 has been concerned with two enzymes in an anaerobic bacterium (cl. kluyverii) viz, imidazole acetylase and hydrogenase, whose exact function and physiological role have not yet been determined.

2. Progress During the Past Six Months:

a) Imidazole Acetylase: This enzyme catalyses the following reaction:

Acetyl CoA + Imidazole → Acetyl - Imidazole + CoA

A simple assay for this enzyme has been devised in which the acetylation of diethylaminoethanethiol by acetyl-phosphate is measured in the presence of phosphotransacetylase, CoA, and Imidazole. Imidazole acetylase has been purified 15 fold and the effect of such parameters as pH, protein concentration, substrate concentration, etc. have been evaluated.

b) Hydrogenase: This enzyme(s) catalyses the oxidation of molecular hydrogen by a wide variety of electron acceptors. Two assay tests are currently employed for measuring hydrogenase activity. The first depends on the reduction of tetrazolium dye, the second, on the reduction of DPN (measured indirectly by coupling with lactic dehydrogenase)

Form No. ORP-2
Oct. 1957

PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
Laboratory of Chemical Pharmacology

Estimated Obligations for FY 1958

Total:	$462,578	
Direct:		$333,733
Reimbursements:		129,845

Form No. CRP-2
Oct. 1957

Serial No. NHI-85
1. Laboratory of Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies with D-Ascorbic Acid

Principal Investigator: Dr. J. J. Burns

Other Investigators: Dr. Peter G. Dayton
Miss Carole Evans

Cooperating Units: Goldwater Memorial Hospital, New York

Man Years: Patient Days: None
Total: One-half year
Professional: One-half year
Other:

Project Description:

General Purpose of Research:

To evaluate the Vitamin C activity of D-ascorbic acid. To investigate the physiological disposition of D-ascorbic acid.

Progress During Past Year:

Previously we reported that D-ascorbic acid is retained by Vitamin C deficient guinea pigs to a considerably lesser extent than L-ascorbic acid, which suggested a possible explanation for its lack of Vitamin C activity! During the past year the Vitamin C activity of D-ascorbic acid has been determined in bioassay experiments under conditions in which equivalent concentrations of the D and L-isomer were maintained in the tissues of guinea pigs. The results obtained in this study show that D-ascorbic acid actually has Vitamin C activity in respect to maintenance of weight and survival of scorbutic guinea pigs. However, the animals receiving D-ascorbic acid showed marked hemorrhagic manifestations upon autopsy, similar to that observed in scorbutic guinea pigs. These results suggest that some of the functions of Vitamin C can be replaced by the D-isomer, but that others are sterio-specific requiring only the L-isomer.

Serial No. NHI-85

Direction of Current Research:

Our finding that D-ascorbic acid has Vitamin C activity in respect to maintenance of weight and survival in scorbutic guinea pigs encouraged us to investigate other functions of Vitamin C which can be replaced by D-ascorbic acid. A project has been initiated in collaboration with Dr. H. M. Fullmer of the NIDH to study the effects of D-ascorbic acid on the development of teeth. This study is of particular interest in view of the fact that changes in dental structures are a sensitive index of Vitamin C activity. Preliminary results obtained in this study show that D-ascorbic acid has an effect similar to L-ascorbic acid for the production of dentine in the teeth of guinea pigs.

Previously we reported that D-ascorbic acid, when administered to guinea pigs and rats, is rapidly excreted in the urine. These results suggest that D-ascorbic acid, unlike L-ascorbic acid, is not reabsorbed by the renal tubule cell. Further studies are planned to compare, in dogs, the renal clearance of D-ascorbic acid and L-ascorbic acid. It is hoped that this study may give information on the mechanisms by which L-ascorbic acid is handled by the kidney.

Previous studies employing labeled D-ascorbic acid suggested that this compound is not absorbed when given orally to rats and guinea pigs. Studies are now underway in collaboration with Dr. Lewis Schanker and Mr. Dominick Tocco to compare the absorption of D and L-ascorbic acid from the gastrointestinal tract. Such a study may point out the mechanism for the absorption of Vitamin C from the gastrointestinal tract.

Incidental Findings of Significance: None

Part B included [X] Yes

Serial No. NHI-85

PHS-NIH
Individual Project Report
Calendar Year 1957

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, J. Biol. Chem., in press.

Honors and Awards relating to this project: None

Form No. ORP-2
Oct. 1957

Serial No. NHI-86
1. Laboratory of Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the Biosynthesis of Cardiotonic Steroids

Principal Investigator: Dr. Elliott Schiffmann

Other Investigators: Dr. Elwood O. Titus

Cooperating Units: None

Man Years:
Total: One and one-half years
Professional: One and one-half years
Other:

Patient Days: None

Project Description:

General Purpose of Research:

Although the cardiac lactones have been used therapeutically for many years, almost nothing is known about their biosynthesis. Several of these steroidal cardiotonic lactones of known structure occur in the parotoid gland of the tropical toad, Bufo marinus, and this gland has provided an easily accessible system in which to study the biogenesis of the cardiac steroids. It is of interest that the toad steroids are almost the only examples of digitalis-like molecules occurring in animals.

Progress During Past Year:

It has previously been demonstrated in this laboratory that cholesterol is a precursor of the cardiac lactone marinobufagin (See Fig. I) and its suberyl arginine conjugate marinobufotoxin. This conversion takes place in the whole animal.

During the past twelve months attempts have been made to elucidate the intermediate steps between cholesterol and the cardiac lactones. Research has proceeded in two directions:

Serial No. NHI-86

A. A search for more efficient systems (preferably in vitro) for the conversion of radioactive cholesterol to marinobufagin. A system yielding a product of high specific activity could be used for the trapping of radioactivity in suspected intermediates.

1. Multiple injections of labeled cholesterol in vivo and counting of the chromatographically isolated marinobufagin have yielded a final product of higher specific activity. Multiple injections of C^{14} acetate can also give a labeled product. (See Table I). This approach does not appear as profitable as in vitro experiments because of the excessive requirements for isotope and difficulties in removing unlabeled endogenous steroids.

2. Although slices and homogenates of the parotoid gland do not convert cholesterol to cardiac lactones, in vitro incubation of radioactive cholesterol with suspensions of freshly expressed venom and chromatography of the isolated steroids has yielded labeled marinobufagin. The specific activity of the product compared favorably with that obtained in whole animal experiments and could be raised to considerably higher values by elimination of the endogenous steroids from the venom preparation. In vitro conversion of cholesterol to marinobufagin by an acetone powder of expressed venom has also been demonstrated. Preparation of the acetone powder removes a portion of the endogenous steroid, but lessens the activity of the preparation (See Table I).

B. Tests with labeled intermediates of hypothetical pathways for the formation of marinobufagin.

1. Transformations at carbon 3 of cholesterol.

Although there are configurational and other differences, the cardiac steroids from the toad bear a formal resemblance to bile acids and may be considered as lactones of doubly unsaturated bile acids. Unlike most bile acids, however, the toad lactones have the same β configuration of the 3 hydroxy substituent as the cholesterol precursor. If it could be demonstrated that the configuration around carbon 3 was retained intact throughout the conversion of cholesterol to marinobufagin, the biosynthetic scheme could be greatly simplified by elimination of possible intermediates. This demonstration could be made with the aid of stably bound hydrogen isotopes, which would be retained on carbon 3 with retention of configuration, but would be lost if the pathway involved a ketonic intermediate at C_3.

Serial No. NHI-86

Radioactive cholesterol-$3H^3$ was prepared by lithium aluminum tritide reduction of Δ5-cholestenone and incubated with toad venom. Data in Table I indicate loss of isotope and, hence, involvement of a ketonic intermediate.

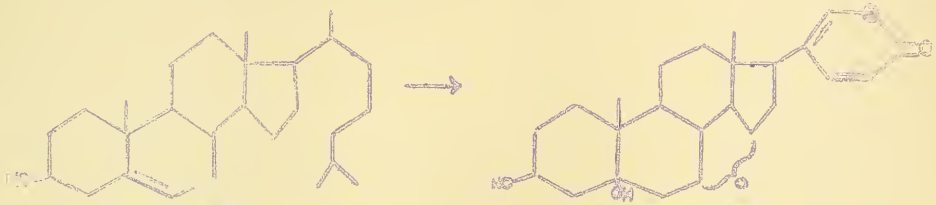
2. Since a ketonic intermediate at C3 seems to be involved and since animal and bacterial enzyme systems are known to reduce 3-keto bile acids to 3 β as well as 3 α hydroxy derivatives, it seemed possible that a 3-keto bile acid might be an intermediate. Since the simplest of these could be readily synthesized from available radioactive intermediates, 3-keto cholanic acid-$24C^{14}$ was prepared and administered to a toad. No significant incorporation of radioactivity into marinobufagin could be demonstrated. Although not conclusive, this result indicates the possibility that hydroxylation and unsaturation reactions precede the oxidative attack on the side chain.

Direction of Current Research:

1. Efforts to find a suitable in vitro system for the biogenesis of cardiac lactones will continue.

2. Venom extracts from toads receiving labeled cholesterol are being fractionated in a search for intermediates.

3. If hydroxylation of the sterol nucleus precedes degradation of the side chain, as it does in the synthesis of the bile acids, 21-hydroxy-14-β-hydroxy-, and 21,14-β-dihydroxycholesterol may be expected to be key intermediates. These compounds are as yet unknown. Their preparation is being undertaken with the aid of the hydroxylating fungi, Ophiobolus herpotrichus and Mucor Griseocyanus.



CHOLESTEROL

MARINOBUFAGIN

Serial No. NHI-86

Table I

Toad Cardiac Lactone Biosynthesis

	System	Substrate	Incubation or Reaction Time	CPM/mg. Marinobufagin
	In vivo	10 uc cholesterol in 5 doses	3 weeks	216
A	In vivo	10 uc acetate in 4 doses	3 weeks	40
	Direct venom In vitro	1 uc cholesterol	2 hours	110
	Acetone powder In vitro	1 uc cholesterol	2 hours	46
B	In vivo	1 uc ketocholanic acid	3 weeks	6.0
	In vivo	10 uc cholesterol-3-T	3 weeks	3.0

Incidental Findings of Significance: None

Part B. included [X] Yes

Serial No. NHI-86

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Siperstein, M. D., Murray, A. W. and Titus, E. O. Biosynthesis of Cardiotonic Sterols from Cholesterol in the Toad, Archives of Biochem. and Biophys., 67, 154 (1957).

Honors and Awards relating to this project: None

Form No. CRP-2
Oct. 1957

Serial No. NHI-87
1. Laboratory of Chemical Pharmacology
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

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: Goldwater Memorial Hospital, New York; Mount Sinai Hospital, New York and Geigy Laboratories, Basel, Switzerland

Man Years: Patient Days: None
Total: One-half
Professional: One-half
Other:

Project Description:

General Purpose of Research:

Butazolidin, 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. Butazolidin 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 Butazolidin 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 Butazolidin, 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.

Progress During Past Year:

Screening Program

Various compounds have been synthesized and tested for anti-inflammatory 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.

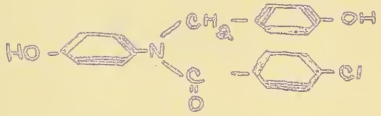
Up to the present, 45 analogues of Butazolidin have been studied in respect to their physiological disposition, their antirheumatic properties in acute gout and rheumatoid arthritis and their effect on urinary excretion of sodium and uric acid. Results obtained so far gives us an idea of what structural features are required in the molecule for the various pharmacological actions of Butazolidin. For instance, substitutionof various groups in the para position of the benzene ring leads to compounds with marked anti-rheumatic and sodium retaining effects. Substitutions in the ortho position also produces marked sodium retention. Substitution in the meta position, however, robs the compound of its sodium retaining effects but preserves some antirheumatic activity. All changes in the butyl side chain results in compounds having potent uricosuric effect but little or no antirheumatic and sodium retaining properties. In general, it has been found that any change in either the benzene rings or butyl side chain which increases the acidity of the molecule increases uricosuria.

These observations on the relationship between chemical structure and pharmacological activity aid in the development of more useful drugs for the treatment of arthritic

diseases. So far in this study, two new drugs have been discovered which are now undergoing extensive clinical trials by various research groups. One drug is Metabolite I, a para hydroxyl metabolite of Butazolidin, which has potent antirheumatic activity in acute gout and rheumatoid arthritis. The other, G-28, a sulfoxide metabolite is the most potent uricosuric agent yet described and appears to be of considerable value for the treatment of chronic tophaceous gout.

Direction of Current Research:

a) Certain compounds (G-29563, G-29200 and G-25899) which have changes in the pyrazolone ring of Butazolidin were found to have potent anti-inflammatory activity in the animal screening tests. G-25963 and G-25899 possessed no anti-inflammatory activity in acute gout and rheumatoid arthritis which demonstrates the difficulty of extrapolating anti-inflammatory data obtained in the animal screening tests to the disease in patients. G-29200 was found, however, to have definite, but mild anti-inflammatory activity in four patients with acute gout. This finding is of considerable interest since the compound has a structure completely different from that of Butazolidin shown as follows:



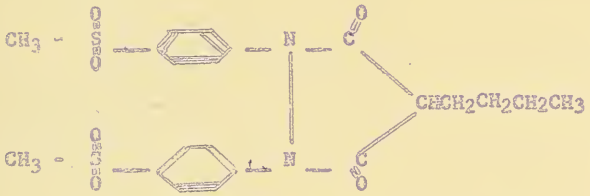
G-29200

For this reason it is planned to study further this compound in acute gout and rheumatoid arthritis. Several other related compounds in this series which also have potent anti-inflammatory activity in the animal screening tests will be investigated.

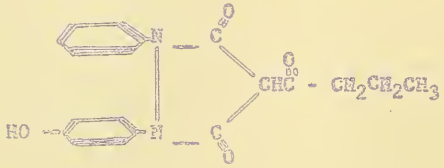
b) More detailed studies are now being carried out to evaluate the uricosuric activity of the sulfoxide metabolite in patients with chronic gout. This study should point out the minimum dosage required for adequate uricosuria and give information on possible side effects which may appear only on prolonged therapy. To date about 35 patients have been administered this drug by our group for periods up to six months and the incidence of side effects have been low. The majority of these patients have severe tophaceous gout which presented problems in management with other uricosuric agents.

c) Further studies are being carried out to evaluate the antirheumatic effects of Metabolite I (p-hydroxyl analogue) in acute gout and rheumatoid arthritis. So far the drug has been administered by Dr. Gutman to 45 patients with acute gout and 10 patients with acute rheumatoid arthritis. These studies have further confirmed the potent antirheumatic activity of this drug reported last year.

d) An analogue of Butazolidin, which has a nitro group in the para position of one of the benzene rings, was found to have antirheumatic, sodium retaining and uricosuric effects. The observation that all three pharmacological activities of Butazolidin are potentiated in this drug agrees with our generalizations that substitutions in the para position enhance antirheumatic and sodium retaining effects and that substitutions which increase the acidity of the drug potentiate uricosuric activity. The para nitro analogue is a stronger acid (pKa 3.2) than Butazolidin (pKa 4.6). In view of the results with this nitro analogue another acidic compound (pKa 2.6) was synthesized with the following structure:



It is planned to evaluate its uricosuric, antirheumatic and sodium retaining properties in patients. A drug which would have both potent antirheumatic and uricosuric activity would be of considerable value for the treatment of acute and chronic gout. In this connection, a compound is also being tested which has the following structure:



This compound has the para hydroxyl group of Metabolite X which enhances antirheumatic activity and a keto group in the side chain which makes it strongly acidic (pK 2.2) so as to potentiate uricosuric activity.

e) Renal clearance studies show that the sulfoxide metabolite has a very potent effect in blocking the tubular secretion of p-aminohippuric acid. For this reason experiments were carried out to see if this compound, like Benamid, is able to maintain plasma levels of penicillin. Preliminary results, indicating that the sulfoxide does have such activity, are sufficiently encouraging to warrant more detailed studies along this line.

f) 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. The possibility that the sulfoxide may be further metabolized to a sulfone is also under investigation. Upon administration of the sulfone to man only a small portion is recovered in urine, suggesting that it may be an important metabolite of the sulfoxide in the body.

g) Previously we reported that an analogue which has a meta hydroxyl group in one of the benzene rings possesses little or no sodium retaining effects, but does have some antirheumatic activity. Further studies are being carried out to evaluate the antirheumatic effect of another analogue which has hydroxyl groups in the meta position of both benzene rings. This compound, like the mono hydroxyl compound, produces little or no sodium retention. Another compound of this type is available which has methyl sulfone groups in the meta positions of both benzene rings. The observation that substitutions in the meta position robs the compounds of sodium retention may be an important lead in finding a new antirheumatic drug.

h) Gastric ulceration is one of the most serious side effects observed in patients receiving Butazolidin. Recent studies now show that it is possible to produce ulceration in animals by giving large doses of the drug. Various analogues of Butazolidin 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, gastro-

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intestinal bleeding was observed in dogs with this compound at a dose level where Butazolidin 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.

Incidental Findings of Significance: None

Part B included [X] Yes

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Burns, J. J., Yü, T. F., Ritterband, A., Perel, J. F., Gutman, A. B. and Brodie, B. B. A Potent New Uricosuric Agent, The Sulfoxide Metabolite of the Phenylbutazone Analogue, G-25671, J. Pharm. and Exptl. Therap., 119, 3 (1957).

Brodie, B. B., Burns, J. J., Paton, B. C., Steele, J. M., Yu, T. F. and Gutman, A. B; Isolation, Identification and Physiological Effects of Phenylbutazone Metabolites, Contemporary Rheumatology, 1956.

Honors and Awards relating to this project: None

Form No. ORP-2
Oct. 1957

Serial No. NHI-88
1. Laboratory of Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies with L-Xylulose

Principal Investigator: Dr. J. J. Burns

Other Investigators: Dr. Peter G. Dayton
Mr. Julian Kanfer
Miss Ruth Gastel

Cooperating Units: NIAMD, National Institutes of Health
Goldwater Memorial Hospital, New York

Man Years:
Total: Two years
Professional: Two years
Other:

Patient Days: None

Project Description:

General Purpose of Research:

To study the biosynthesis and metabolism of L-xylulose, the sugar excreted by patients with essential pentosuria.

Progress During Past Year:

a) An active enzyme system has been found in the soluble fraction of rat kidney which decarboxylates L-gulonic acid forming L-xylulose. Evidence was also obtained for xylitol as a product of this reaction. The reaction products were identified by carrier dilution techniques, paper chromatography and specific enzymatic assay methods.

b) Evidence was obtained in vivo for the following pathway of metabolism of L-gulonic acid in rats and guinea pigs.

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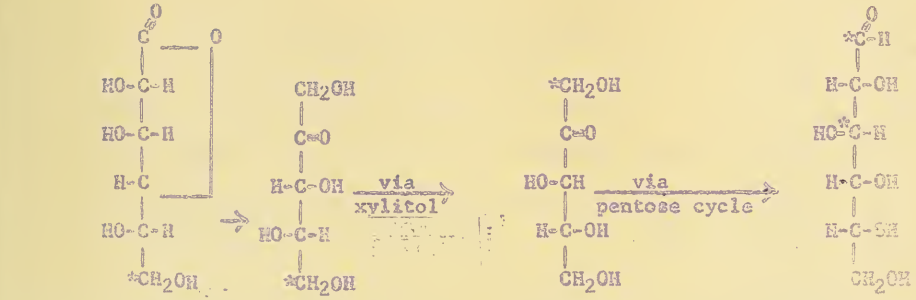

In these experiments the incorporation of a doubly labeled L-gulonic acid tracer (carbon-1 labeled with C^{14} and carbon-6 labeled with C^{13}) into glucose of the liver glycogen was measured. The results obtained are in accord with the predictions based on this pathway. In addition, the data showed no detectable conversion of L-gulonic acid back to glucose via D-glucuronic acid.

Direction of Current Research:

a) Further studies are being carried out to purify the enzyme system in kidney which converts L-gulonic acid to L-xylulose. So far about a 20 fold purification has been achieved and evidence has also been found suggesting that there are two distinct reactions involved - an oxidation step requiring DPN and a decarboxylation reaction.

b) The occurrence in other tissues of the L-gulonic acid decarboxylating enzyme will be investigated. It appears that marked differences exist in vitro in the metabolism of this compound in the rat and guinea pig. Guinea pig kidney, unlike that of the rat, possess little activity to decarboxylate L-gulonic acid; whereas guinea pig liver is considerably more active than rat liver. Studies of this species difference will be of interest in view of our finding that rats and guinea pigs differ markedly in their ability to convert L-gulonic acid to L-ascorbic acid.

c) Studies are being carried out to determine whether D-glucuronic acid, like L-gulonic acid, is also metabolized in the body via L-xylulose formation. In these experiments the incorporation of a double labeled D-glucuronic acid tracer (carbon-1 labeled with C^{13} and carbon-6 labeled with C^{14}) into liver glycogen is being measured.

Incidental Findings of Significance: None

Part B included [X] Yes

Serial No. NHI-88

PHS-NIH
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Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Burns, J. J. and Kanfer, J. Formation of L-Xylulose from L-Gulonolactone in Rat Kidney, J. Amer. Chem. Soc., 79, 3604 (1957).

Burns, J. J., Dayton, P. G. and Eisenberg, F., Jr. Metabolism of L-Gulonolactone in Rats via Pentose Formation, Biochim. et Biophys., 25, 647 (1957).

Honors and Awards relating to this project: None

Form No. CRP-2
Oct. 1957

Serial No. NHI-S9
1. Laboratory of Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Applications of Spectrophotofluorometry

Principal Investigator: Dr. Daniel E. Duggan

Other Investigators: None

Cooperating Units: None

Man Years: Patient Days: None
Total: One year
Professional:
Other:

Project Description:

General Purpose of Research:

The original purpose of this research activity, a general survey of the applicability of spectrophotofluorometric techniques to biochemical and pharmacological problems, has been realized and activity subsequently directed toward the more specific purpose of isolating and characterizing an unknown fluorescent constituent of normal mammalian tissue.

Progress During Past Year:

A. On the basis of physical measurements (partition constants, paper electrophoretic mobility, behavior on ion-exchange media) the unknown fluorescent material has been tentatively characterized as a non-amphoteric, weakly acidic substance of pKa 9-10. Various chemical studies have indicated that this material occurs in tissue in the form of an extremely polar, alkali-labile complex which is insensitive to all of the various enzymes employed in attempted cleavage (acid and alkaline phosphatase, sulfatase, diastase, papain, etc.).

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B. Combinations of ion-exchange chromatrography, magnesol adsorption, and paper chromatography have yielded a relatively pure concentrate which appears to consist of three fluorescent compounds, whose activation and fluorescence spectra show only minor differences. All absorb light at 360-370 mu and fluorece maximally at 460 mu.

C. The behavior of these materials toward various chemical reagents has definitely excluded as possible structures those of the flavin and pterin groups, the only known compounds whose fluorescence characteristics approximate those of the unknown.

Direction of Current Research:

Current research activity is directed toward two immediate goals: (a) Adequate separation and purification of each of the fluorescent components as to allow definitive infra-red and ultraviolet absorption spectra. (b) The stockpiling of crude concentrate with the ultimate aim of obtaining pure material for chemical investigation.

Incidental Findings of Significance: None

Part B included [X] Yes

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Individual Project Report
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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Duggan, D. E., Bowman, R. L., Brodie, B. B. and Udenfriend, S. A Spectrophotofluorometric Study of Compounds of Biological Interest, Arch. Biochem. Biophys., 68, 1-14 (1957).

Udenfriend, S., Duggan, D. E., Vasta, B. M. and Brodie, B. B. A Spectrophotofluorometric Study of Organic Compounds of Pharmacological Interest, J. Pharm. and Exptl. Therap., 120, 26-32 (1957).

Honors and Awards relating to this project: None

Form No. CRP-2
Oct. 1957

Serial No. NHI-90
1. Laboratory of Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Comparative Effects of Digitalis on Contractile Force of Normal and Failing Heart Muscle

Principal Investigator: Dr. Marion deV. Cotten

Other Investigators: Miss Phyllis E. Stopp
Mr. William M. Butler, Jr.
Mr. James A. Watts

Cooperating Units: None

Man Years:
Total: Two years
Professional: Two years
Other:

Patient Days: None

Project Description:

General Purpose of Research:

It has been generally accepted that digitalis glycosides have a pronounced stimulant effect on failing heart muscle both *in vivo* and *in vitro*, but have either no effect or a moderate depressant action on normal heart muscle. In normal animals and humans, digitalis decreases cardiac output and stroke work while in heart failure cardiac output and work are usually increased substantially. Similarly, digitalis has been reported to lack an effect on non-failing isolated heart muscle, but to have a pronounced stimulant action on failing isolated heart muscle. These findings imply that digitalis corrects some specific derrangement in failing cardiac muscle which is absent in normal heart muscle. Several types of experiments have been conducted to reasses the validity of these concepts since it had been observed that digitalis glycosides produce a substantial increase in ventricular contractile force in normal, unanesthetized dogs.

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Progress During Past Year:

Digitalis increases moderately the contractile force in dogs under the following circumstances: (1) unanesthetized, (2) anesthetized with either barbiturates or morphine-chloralose, (3) following surgical removal of sympathetic ganglia from the stellate to the sixth thoracic ganglia, (4) following bilateral adrenalectomy, (5) following bilateral vagotomy, (6) following bilateral cardiac sympathectomy, bilateral adrenalectomy, and bilateral vagotomy, (7) following hepatectomy, (8) following nephrectomy, (9) following section of the spinal cord at the second cervical vertebra, (10) following large doses of hexamethonium and (11) after the cardiac stimulant effects of epinephrine, norepinephrine and isoprotevenol are blocked completely by dibenzyline or phentolamine. Digitalis also evokes a moderate increase in contractile force in anesthetized normal cats, rabbits and in the monkey.

In the anesthetized dog, digitalis increases left ventricular contractile force, blood pressure and systemic peripheral resistance and decreases systemic output, stroke volume, heart rate and left atrial pressure. Calculations of left ventricular stroke work show that work is decreased slightly by digitalis glycosides in normal anesthetized dogs. However, a decrease in systemic output in the presence of an increase in contractile force and blood pressure and a decrease in heart rate and left atrial pressure indicated that there was a decrease in the volume of blood in the central vein and lungs with a shift to peripheral vascular areas. When left atrial pressure was prevented from changing during the actions of digitalis, there was a moderate increase in system output, stroke volume, systemic peripheral resistance, left ventricular stroke work and left ventricular contractile force while the heart rate was decreased. These results indicate that the normal heart is stimulated by digitalis but that this stimulation is not evident in the intact animal since the volume of blood available to fill the ventricles is diminished. Further evidence of the cardiac stimulant action of digitalis in normal dogs was obtained by comparing ventricular function curves before and after digitalis. After digitalis, there was a significant increase in the ventricular function curve. Thus, digitalis enables the normal heart to produce greater amounts of stroke work at comparable levels of ventricular filling pressure.

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Additional experiments conducted in the heart lung preparation (HLP) demonstrated that digitalis evokes a moderate increase in contractile force both in the "non-failing HLP" and the failing HLP. Whether or not systemic output and stroke work are increased by digitalis in the "non-failing HLP" depends upon the level of ventricular filling pressure. At low ventricular filling pressures there is little change in output or work, but when left ventricular filling pressure is maintained at levels obtaining in the intact animal, output and work are increased while left atrial pressure falls.

Experiments with the isolated papillary muscle of the cat demonstrated that both the failing and non-failing papillary muscle are stimulated by digitalis.

The above data indicate that the effect of digitalis on the contractility of heart muscle is qualitatively similar in normal and failing heart muscle, both *in vivo* and *in vitro*.

Direction of Current Research:

This project is complete and no additional experiments are planned.

Incidental Findings of Significance: None

Part B included [X] Yes

Serial No. NHI-90

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Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Cotten, N. deV. and Stopp, Phyllis E. Action of Digitalis on the Non-Failing Heart of the Dog, Am. J. Physiol., in press.

Honors and Awards relating to this project: None

Form No. CRP-2
Oct. 1957

Serial No. NHI-91
1. Laboratory of Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Reversal of the Cardiac Effects of Sympathomimetic Amines by Digitalis Glycosides During Hypothermia

Principal Investigator: Dr. Marion deV. Cotten

Other Investigators: Dr. Theodore Cooper
Miss Phyllis E. Stopp
Mr. William M. Butler, Jr.
Mr. James A. Watts

Cooperating Units: Surgery Branch, National Heart Institute

Man Years: Patient Days: None
Total: Two years
Professional: Two years
Other:

Project Description:

General Purpose of Research:

These experiments were designed to compare the actions of drugs at normal body temperature with those at body temperatures of 25-30° C. Special emphasis was placed upon possible differences in the inter-actions of various drug groups during hypothermia in view of the clinical importance of such infotmation.

Progress During Past Year:

The administration of epinephrine, norepinephrine and isoproterenol produces substantial increments in cardiac contractile force at normal body temperature and at a body temperature of 30° C. Similarly, the digitalis glycosides such as ouabain and digoxin also produce substantial increments in contractile force at normal body temperature and at 30° C. The administration of ouabain or digoxin at normal body temperature does not alter, in any way, the cardiac or blood

pressure responses to the three sympathomimetic amines. However, following the administration of ouabain or digoxin and cooling of the animals to 30° C, the cardiac stimulant effects of epinephrine, norepinephrine and isoproterenol are either completely blocked or, in most instances, reversed. The pressor responses to epinephrine and norepinephrine are completely blocked in most instances. The effects of other sympathomimetic amines such as ephedrine and methamphetamine are blocked or reversed at a body temperature of 30° C following digoxin or ouabain. The body temperature at which the glycoside is administered does not appear to alter the development of this blockade, being equally effective when administered at normal body temperature with subsequent cooling or when administered during hypothermia. On the basis of a limited number of experiments, re-warming the hypothermic animals to normal body temperature appears to re-establish the cardiac stimulant effects of the sympathomimetic amines while subsequent re-cooling again results in blockade or reversal of the cardiac stimulant effects of the amines.

Direction of Current Research:

The project is complete and no additional experiments are planned.

Incidental Findings of Significance: None

Part B included / X / No

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Oct. 1957

Serial No. NHI-92
1. Laboratory of Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies with L-Ascorbic Acid

Principal Investigator: Dr. J. J. Burns

Other Investigators: Dr. Peter G. Dayton
Dr. Nicholas Papadopoulos
Miss Natalie Trousof
Miss Ruth Gastel

Cooperating Units: Laboratory of Biochemistry and Metabolism, NIAMD
Goldwater Memorial Hospital, New York

Man Years: Patient Days: None
Total: One and one-half years.
Professional: One and one-half years.
Other:

Project Description:

General Purpose of Research:

To study the enzyme systems 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.

Progress During Past Year:

a) Man, monkey and guinea pig are the only mammals known to be unable to synthesize L-ascorbic acid. It is for this reason that they require the vitamin in their diet to prevent scurvy. Isotopic studies reported previously have shown that the rat, a typical animal species that can exist without this vitamin, synthesizes it in its liver by a three step reaction as follows:

D-glucose → D-glucuronic acid → L-gulonic acid → L-ascorbic acid

Man, monkey and guinea pig can carry out the first two reactions, but lack the enzyme system required for the conversion of L-gulonic acid to L-ascorbic acid. It is this "missing step"

which explain why man, monkey and guinea pig require Vitamin C in their diet.

b) Previously we reported that the main route of metabolism of L-ascorbic acid in guinea pigs involves extensive oxidation of the carbon chain to CO_2. In studies carried out in collaboration with Dr. Frank Eisenberg, Jr. of NIAMD information has now been obtained on the possible intermediates involved in the breakdown of the vitamin. In these experiments the incorporation and distribution of C^{14} into glucose of liver glycogen was compared following administration to guinea pigs of both carbon-1 and carbon-6 labeled L-ascorbic acid tracers. The results obtained are in agreement with a pathway of metabolism of the vitamin via decarboxylation yielding pentoses. Previous studies from our group have pointed out an active enzyme in liver and kidney for decarboxylation of L-ascorbic acid. Recently C. G. King and coworkers have shown that L-xylose is actually a product of such a reaction. The results obtained in our experiments further suggest L-glyceraldehyde as an intermediate in the metabolism of L-ascorbic acid. This is of interest, since we had found previously that this triose is also formed from L-sorbose in the body, a sugar which is structurally closely related to L-ascorbic acid.

Direction of Current Research:

a) Further studies will be continued on the enzyme system in rat liver microsomes involved in the conversion of L-gulonic acid to L-ascorbic acid. The properties of this system will be compared with those of the one found in rat kidney which converts L-gulonic acid to L-xylulose. In particular, an attempt will be made to determine if these two enzymatic reactions share 3-keto-L-gulonic acid as a common intermediate.

b) Further investigations will be continued with differently labeled L-ascorbic acid tracers to evaluate the importance of pentose and triose formation in the over-all metabolism of the vitamin.

c) Little information is available on the biosynthesis and metabolism of L-ascorbic acid in bacteria. It is planned to investigate this point in several representative micro-organisms. The experimental procedures for this study have now been perfected.

d) 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 con-

centration as in the liver. Studies will be carried out to determine the mechanism of binding of the vitamin to tissue components.

e) Evidence is available for the enzymes involved in the biosynthesis of ascorbic acid except for the conversion of glucose to glucuronic acid. An interesting lead in this direction comes from the recent observation by others for an active enzyme in rat kidney which converts inositol to glucuronic acid. This observation, taken together with the finding that glucose is converted to inositol, suggests that this compound may be the actual intermediate involved in the biosynthesis of glucuronic acid from glucose. In preliminary experiments carried out in collaboration with Dr. Bernard Agranoff, NIMDB, it has been found that administration of tritium labeled inositol to rats gives rise to significant labeling in urinary glucuronic acid and gulonic acid. These results have encouraged us to search for the enzymes involved in the following reaction: D-glucose → inositol → glucuronic acid.

Incidental Findings of Significance: None

Part B Included [X] Yes

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PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Burns, J. J. and Evans, C. Synthesis of L-Ascorbic Acid in the Rat from D-Glucuronolactone and L-Gulonolactone, J. Biol. Chem., 223, 897 (1956).

Burns, J. J. Missing Step in Man, Monkey and Guinea Pig Required for the Biosynthesis of L-Ascorbic Acid, Nature, 180, 553 (1957).

Hellman, L. and Burns, J. J. Metabolism of L-Ascorbic Acid-1-C^{14} in Man, J. Biol. Chem., (in press).

Dayton, P. G. The Synthesis of L-Ascorbic-C_6^{14} and L-Ascorbic-6-C^{14} Acid, J. of Organic Chem., 21, 1535 (1956).

Dayton, P. G. Fate of 2-Keto-L-Gulonic Acid in Rat and Guinea Pig, Proc. Soc. Exper. Biol. and Med., 94 286 (1957).

Burns, J. J., Peyser, P. and Moltz, A. Missing Step in Guinea Pig Required for the Biosynthesis of L-Ascorbic Acid, Science, 124, 1148 (1956).

Honors and Awards relating to this project: None

Form No. CRI-2
Oct. 1957

Serial No. NHI-99
1. Laboratory of Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the Physiological Role of Phospholipids

Principal Investigator: Dr. Herbert Weiss

Other Investigators: Dr. Elwood O. Titus
Miss Anne W. Murray

Cooperating Units: None

Man Years: Patient Days: None
Total: Two years
Professional: One and one-fourth years
Other: Three-fourths

Project Description:

General Purpose of Research:

Although the physiological role of the phospholipids is still obscure, there are a number of indications that their metabolism is concerned with the functioning of cellular membranes. The lytic effects on erythrocytes and mitochondria and the digitalis-like action of lysolecithins are suggestive in this respect. Evidence from other laboratories indicates that the turnover of specific phospholipids may be associated with the release of protein hormones from secreting cells. It has been suggested in the literature that the release of pharmacodynamic agents such as histamine from mast cells may result from changes in cell permeability induced by lipids produced in the cell.

It is the general purpose of this project to examine the metabolism of phospholipids in the hope of clarifying their physiological role. If these substances are implicated in the control of the passage of electrolytes through cellular membranes, or in the release of pharmacodynamic agents from secreting cells, they would be significant factors in maintaining the normal function of the cardiovascular system.

Progress During Past Year:

1. Enzymatic Studies

In the course of purification of cabbage phospholipase D, a soluble enzyme which detaches choline from lecithin, it was observed that the enzyme required calcium ion. Although the enzyme released both choline and ethanolamine from crude vegetable lecithin (the ethanolamine apparently coming from a phosphatidylethanolamine contaminant) it was without effect on purified or synthetic lecithin.

Fractionation of the crude vegetable lipid preparation by chromatography and precipitation with solvents yielded a fraction which, when added to pure lecithin, permitted the enzyme to function. This "activator" appears to be a very non-polar lipid, chromatographically similar to phosphatidic acid. It has not yet been isolated in sufficiently pure form to permit identification. Preliminary evidence indicates that the "activator" is not necessary for the action of cabbage phospholipase D on lysolecithin. The mechanism of action of the activator is not yet known. It apparently does not act by solubilizing the substrate, since a variety of detergent and surface active agents, which enhance the solubility of lecithin are without effect on the enzyme system.

Other laboratories have reported the presence of this enzyme in the particulate matter from cabbage cells. These preparations are active on synthetic lecithins, but only after treatment with ether, which apparently lyses the particles and releases an activating substance. Somewhat similar phenomena have now been reported with several other phospholipases that attack lecithin in other positions and it is possible that the presence of a lipoidal "activator" is a general requirement for the activity of enzymes that degrade lecithin.

2. Studies of Phospholipid Turnover with Radioactive Phosphorus

It has been postulated by some workers that membrane phospholipids may serve as "carriers" in the active transport of cations through cellular membranes. One means of providing energy for these processes would be by synthesis of carrier on one side of a membrane and its breakdown on the other, in which case the turnover of the carrier lipid should vary as the pumped flux of cations is altered.

Since there are not sufficient data on the metabolism of phospholipids to permit a critical examination of the various hypotheses concerning the role of phospholipids in membranes, a study of the rate of incorporation of P^{32} into red cell phospholipids was undertaken. The red cell was chosen because phospholipid occurs only in the membrane and because the pumped fluxes of K^+ into and Na^+ out of the cell may be varied experimentally.

It has proved possible to measure the incorporation of P^{32} from inorganic phosphate into 5 phospholipids individually. Of these, only one, a very non-polar substance chromatographically similar to phosphatidic acid, is significantly labeled by incubation in vitro. Incorporation of P^{32} into this fraction is decreased by ouabain, a substance which is known to decrease the ability of the red cell to accumulate K^+ against a concentration gradient. See below.

	Counts/min. P after incubation	
Normal Human RBC	Control	10^{-6} M Ouabain
	213	61
RBC accumulating K^+ after loss by chilling	530	275

Preliminary studies with rabbit kidney cortex slices indicate that significant labeling of all the phospholipids occurs upon incubation in P^{32} labeled buffers. The presence of the potent Na^+ retaining steroid, 2-methyl-9 α -fluoro tetrahydrocortisone acetate, is without effect on the phospholipid turnover.

Direction of Current Research:

Although neither the phospholipase D "activator" nor the rapidly turning over component of red cells have been identified, there are strikingly similar chromatographic properties in both. Other laboratories have reported that, whereas intact tissues incorporate inorganic P^{32} labeled phosphate generally into phospholipids, homogenates will incorporate most of the isotope into a rapidly turning over phospholipid with properties very similar to the substances under investigation in these laboratories. This material has been reported to be chromatographically similar to phosphatidic acid, but of unknown structure.

It appears from these and other data that some of the lesser known, very non-polar phospholipids may be important in regulating the metabolism of phospholipids and in the response of cells to drugs.

The structure of these non-polar components will be determined and further studies of their function carried out.

Further studies of phospholipid turnover in relation to cation transport will be made. It is possible that turnover rates are too low to be directly related to cation pumping, but insufficient data are available for an adequate test of the hypotheses.

Studies on the role of lysolecithin and the origin and fate of the aldehyde containing precursors of this substance are to be undertaken. The immediate goal of these studies will be to determine whether enzymatic formation of lysolecithin can serve as a mechanism by which cells can alter membrane permeability and release components such as histamine or norepinephrine.

Incidental Findings of Significance: None

Part B included /X/ No

Form No. PHS-2
Oct. 1957

Serial No. NHI-94
1. Laboratory of Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on Connective Tissue Mucopolysaccharides

Principal Investigator: Dr. Nicholas Papadopoulos

Other Investigators: Dr. J. J. Burns

Cooperating Units: None

Man Years: Patient Days: None
Total: One-half year
Professional: One-half year
Other:

Project Description:

General Purpose of Research:

To study the origin of D-glucuronic acid in mucopolysaccharides of connective tissue.

Progress During Past Year:

The origin of glucuronic acid in mucopolysaccharides (MPS) of connective tissue such as chondroitin sulfate and hyaluronic acid is unknown. Previous investigators were unable to show significant incorporation of exogenous glucuronic acid into connective tissue of adult guinea pigs. Since the rate of connective tissue formation in the adult animal is very slow, little incorporation of glucuronic acid would be expected, thus their findings are inconclusive.

In view of the recent findings by our group of a role for glucuronic acid as a precursor of L-gulonic acid, L-ascorbic acid and L-xylulose, we considered it of importance to further investigate whether exogenous glucuronic acid could serve as a precursor for MPS of connective tissue. Large amounts of D-glucuronolactone-6-C^{14} were administered over a period of 2½ days to actively growing rats, forming new connective tissue.

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In addition, experiments were carried out in guinea pigs after the subcutaneous injection of carrageenin that induces the formation of large amounts of granuloma tissue rich in MPS. The MPS fraction was isolated from a pooled sample of connective tissues obtained from skin, tail tendon, rib cartilage, trachea and nasal septum, and the incorporation of C^{14} determined.

Results showed no significant incorporation of the exogenous glucuronic acid into the MPS of the above mentioned connective tissues. The small amounts of C^{14} found in these tissues could be due to fixation of labeled CO_2 formed in the metabolism of glucuronic acid. Control experiments were carried out under the same conditions with glucose-6-C^{14} and showed a ten fold greater incorporation of C^{14} than obtained with glucuronic acid.

Direction of Current Research:

a) Our finding that exogenous glucuronic acid does not serve as a direct precursor of glucuronic acid of MPS of newly formed connective tissue, as well as the finding by other investigators that exogenous glucuronic acid does not serve for the formation of glucuronides, suggests a different pathway for the origin of endogenous glucuronic acid. Such a pathway, utilizing the UDPG system, has already been suggested and a new one with inositol as intermediate in the conversion of glucose to glucuronic acid is now under investigation.

b) L-Ascorbic acid is necessary for the formation of connective tissue. Its role in the formation of carrageenin induced granuloma tissue will be investigated. In addition, the role of D-ascorbic acid will be investigated in the same tissue, since our group has found that D-ascorbic acid has some Vitamin C activity.

Incidental Findings of Significance: None

Part B included [X] No

Form No. CRP-2
Oct. 1957

Serial No. NHI-95
1. Laboratory of Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Intravenous Anesthetics

Principal Investigator: Dr. J. J. Burns

Other Investigators: Dr. Peter G. Dayton
Miss Dolores Taller

Cooperating Units: Goldwater Memorial Hospital, New York
College of Physicians and Surgeons, Columbia University, New York

Man Years: Patient Days: None
Total: One-half year
Professional: One-half year
Other:

Project Description:

General Purpose of Research:

A study of the physiological disposition and intermediary metabolism of various barbiturates is intended to derive fundamental information concerning the pharmacology of intravenous anesthetics and to provide direction for the development of better 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.).

Progress During Past Year:

Previously we reported that the various intravenous anesthetics used in the operating room including Pentothal,

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Surital, Evipal and Kemithal all owe their short duration of action, not to rapid metabolism, but to their rapid accumulation in body fat. Recently Methitural has been introduced as a new ultra-short acting intravenous anesthetic. The compound has a thioether group in the alkyl side chain shown as follows:

Methitural has been claimed by others to be an ultra-short acting anesthetic because of rapid metabolism based on experiments in rats. However, studies in man point out that the compound is metabolized slowly like Pentothal and owes its short duration of action to rapid accumulation in fat. Methitural, therefore, has the same inherent disadvantage of Pentothal in that patients receiving large doses of the drug have a prolonged hypnotic effect due to slow release of the drug from its depot in fat.

Direction of Current Research:

The findings obtained in our studies show some of the limitations of intravenous anesthetics belonging to the barbiturate series. For instance, they owe their ultra-short action to accumulation in fat and not too rapid metabolism. For this reason a study is now planned with a new intravenous anesthetic which has a chemical structure entirely different from the agents now used. This compound is a phenoxyacetic acid derivative (G-29505) which produces suitable anesthesia with rapid duration of action. In addition, this compound is reported to have little or no effect on respiration, one of the more serious side effects of the barbiturate anesthetics.

Incidental Findings of Significance: None

Part B included [X] Yes

Serial No. NHT-95

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Mark, L. C., Burns, J. J., Campomanes, C. I., Ngai, S. H., Trousof, N., Papper, E. M. and Brodie, B. B. The Passage of Thiopental into Brain, J. Pharm. and Exptl. Therap., 119, 1 (1957).

Honors and Awards relating to this project: None

Form No. ORP-2
Oct. 1957

Serial No. NHL-96
1. Laboratory of Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on a New Pathway of Glucose Metabolism

Principal Investigator: Dr. J. J. Burns

Other Investigators: Dr. Peter G. Dayton
Miss Carole Evans
Mr. Arnold Moltz

Cooperating Units: Goldwater Memorial Hospital, New York

Man Years: Patient Days: None
Total: One and one-half years.
Professional: One and one-half years.
Other:

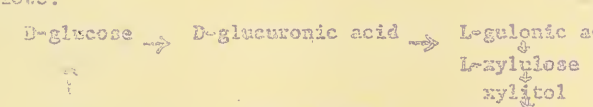
Project Description:

General Purpose of Research:

To investigate new pathways of glucose metabolism in animals.

Progress During Past Year:

a) In recent years there has been considerable interest in pathways of glucose metabolism in animals other than via the classical glycolitic scheme. The best demonstrated example is the hexose monophosphate shunt which leads to the synthesis of pentoses via the intermediate formation of D-gluconic acid. In the course of our studies on the biosynthesis of ascorbic acid, observations have been made which suggest still another pathway of glucose metabolism; the glucuronate shunt shown as follows:



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The key step in this pathway was our finding that L-gulonic acid is converted to L-xylulose. Evidence for the occurrence of all the other reactions have been obtained both in our laboratory and by other investigators. This pathway of glucose metabolism is of importance for the following reasons: (1) It leads to the synthesis of L-ascorbic acid from L-gulonic acid in animals such as the rat. (See project report on L-ascorbic acid). (2) It explains the origin of L-xylulose, the sugar excreted by patients with essential pentosuria. (See project report on L-xylulose). (3) Preliminary results suggest that this scheme may play a role in the biosynthesis of the D-ribose, the important pentose found in nucleic acid. Significant incorporation of C^{14} into ribose of nucleic acid has been found after administration of labeled L-gulonic acid to rats. It is of interest that the labeling pattern observed in ribose of nucleic acid following administration of differently labeled glucose to rats is in agreement with that predicted by this new pathway.

b) Of considerable interest is that various drugs markedly stimulate the metabolism of glucose by this pathway. The effect of these drugs does not involve glucuronide formation, but it appears to be a response on the part of the body to foreign compounds not hitherto reported. There apparently exists a hormonal control over this phenomenon since the drug effect disappears in hypophysectomized rats.

Direction of Current Research:

a) Further studies will be carried out to determine the importance of this new pathway in the synthesis of ribose of nucleic acid.

b) Experiments will be carried out to quantitate the importance of this new pathway in the over-all metabolism of glucose in drug treated animals. For this reason, it is planned to compare the incorporation of variously labeled glucose tracers into liver glycogen of normal and drug treated rats.

c) Further studies will be carried out to define the structural specificity for the effect of drugs on glucose metabolism. For instance, it is planned to investigate whether lipoid solubility and acidity are important. Various analogues in the phenylbutazone series which have markedly different pKa are being studied. In connection with this study a method is being developed for the determination of free glucuronic acid.

Incidental Findings of Significance: None

Part B included [X] Yes

Serial No. NHI-96

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Individual Project Report
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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Burns, J. J., Evans, C. and Trousof, N. The Stimulatory Effect of Barbital on Urinary Excretion of L-Ascorbic Acid and Non-Conjugated D-Glucuronic Acid, J. Biol. Chem., 227, 785 (1957).

Burns, J. J. Biosynthesis of L-Gulonic Acid in Rats and Guinea Pigs, J. Amer. Chem. Soc., 79, 1257 (1957).

Honors and Awards relating to this project: None

Form No. ORP-2
Oct. 1957

Serial No. NHI-97
1. Laboratory of Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies with Muscular Relaxants

Principal Investigator: Dr. J. J. Burns

Other Investigators: Dr. Allan Conney
Miss Natalie Trousof

Cooperating Units: None

Man Years: Patient Days: None
Total: One and one-half years.
Professional: One and one-half years.
Other:

Project Description:

General Purpose of Research:

There is considerable need in clinical medicine for an effective muscular relaxant drug for treatment of multiple sclerosis, cerebral palsy, poliomyelitis and other diseases associated with peripheral muscular spasms. Recently Flexin has been introduced as a new muscular relaxant drug which is thought to act centrally like mephenesin. It is planned to investigate the physiological disposition and metabolic fate of Flexin.

Progress During Past Year:

Flexin is metabolized in man by substitution of a hydroxyl group for the amino group as follows:

FLEXIN HYDROXYL METABOLITE

It is of particular interest that the hydroxyl metabolite has muscular relaxant properties similar to that of the parent drug. Studies on the physiological disposition of the metabolite have shown that it has an advantage over Flexin in being rapidly and completely absorbed. The poor and erratic absorption of Flexin accounts for some of the difficulty in controlling its therapeutic response in patients. Extensive clinical studies have been carried out by several groups which show that the metabolite is actually a more effective muscular relaxant drug than Flexin in the treatment of spastic diseases. There is, indeed, a good possibility that the hydroxyl metabolite will replace Flexin in clinical practice.

Direction of Current Research:

The conversion of the amino group of Flexin to a hydroxyl group represents a type of reaction in drug metabolism which has not been studied previously. Studies are now underway to investigate the enzymes involved in this reaction. Since there is some evidence that the benzoxazole ring in Flexin is cleaved in the body it is planned to study the enzymes involved in this reaction.

Incidental Findings of Significance: None

Part B included [X] No

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Oct. 1957

Serial No. NHI-98
1. Laboratory of Chemical Pharmacology
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Isolation of Cardiotonic Substances from Mammalian Tissues

Principal Investigator: Dr. Elwood O. Titus

Other Investigators: Dr. Herbert Weiss
Dr. Stephen Hajdu
Mr. Herbert Spiegel

Cooperating Units: Laboratory of Kidney and Electrolyte Metabolism

Man Years:
Total: One and one-fourth years
Professional: Three-fourths
Other: One-half year

Patient Days: None

Project Description:

General Purpose of Research:

Earlier reports have discussed evidence from the literature 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 these laboratories the use of a bioassay based on the staircase phenomenon in the frog heart has disclosed the presence of a water soluble active factor in beef adrenal medulla. This has been identified as β -monopalmitoyl - L- α -glyceryl-phosphoryl choline (palmitoyl lysolecithin). Similar substances have been found in lesser amounts in beef heart, liver and plasma. In most tissues the lysolecithins apparently occur largely as inactive molecules containing a long chain fatty aldehyde bound by way of a labile hemiacetal bond to the active lysolecithin.

Evidence has also been obtained for the existence of non-polar ether soluble substances that can affect the frog heart in a manner resembling digitalis. These occur in heart and serum and probably in other tissues. Their isolation and characterization are the immediate goals of the project.

Progress During Past Year:

The isolation of the ether soluble cardiotonic factors has been continued. Active material has been obtained from both rabbit and calf heart by prolonged extraction with hot ethanol and column chromatography of the acidic substances from the ethanol extracts. The active factors, which differ in rabbit and calf, appear to be acids of unknown structure containing approximately 18 to 20 carbon atoms.

Chromatographic comparisons and color tests involving the formation of complexes with dyes indicate that the acids are not identical with either of the two fatty acid derivatives, $\Delta^{3,4}$cis octadecenoic acid and $\Delta^{11,12}$cis octadecenoic acid (cis-vaccenic acid) which have previously been reported to have pharmacological activity. These latter substances have been isolated from serum. They are known to be strongly hemolytic and to act as stimulants of smooth muscle. In comparative tests carried out by Dr. Hajdu (Laboratory of Kidney and Electrolyte Metabolism) the commonly occurring oleic acid ($\Delta^{9,10}$cis octadecenoic acid) had only evanescent effects on the staircase phenomenon in the frog heart. Its isomer, cis-vaccenic acid, on the other hand, appeared to be rather firmly bound to the heart, and in concentrations of approximately 30 gamma per ml. exhibited an effect on the staircase similar to that of the unknown substances from heart.

Preliminary data indicate that the substance from rabbit heart may be chemically similar to cis-vaccenic acid, but with a higher molecular weight. The factor from calf heart appears to be more complex, and may contain a cyclic structure.

Direction of Current Research:

The isolation and identification of the ether soluble factors from heart will be completed. Since the only two species thus far examined have yielded substances with similar biological and chromatographic properties, but different structures, an effort will be made to characterize cardioactive acidic lipids from a variety of mammalian species.

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It appears from preliminary results in this laboratory and from scattered observations elsewhere that relatively minor alterations in the position of double bonds in fatty acids can yield compounds which markedly affect membrane function. Two isomers of oleic acid have been found to be strongly hemolytic and to stimulate smooth muscle preparations. One of these has also proved active in the frog heart assay. A more complete analysis of the effects of structure on pharmacological activity is being contemplated.

Incidental Findings of Significance: None

Part B included /X/ Yes

Serial No. NHI-98

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Titus, E. O., Weiss, H. and Hajdu, Stephen, The Isolation of a Cardiac Active Principle from Mammalian Tissue, Science, 124, 1205 (1957).

Hajdu, Stephen, Weiss, H. and Titus, E. O., The Isolation of a Cardiac Active Principle from Mammalian Tissue, J. Pharm. and Exptl. Therap., 120, 99 (1957).

Honors and Awards relating to this project: None

Form No. ORP-2
Oct. 1957

Serial No. NHI-99
1. Chemical Pharmacology
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A

Project Title: Studies on the Roles of Serotonin and Norepinephrine in Brain Function

Principal Investigators: Dr. Donald F. Bogdanski; Dr. Parkhurst A. Shore

Other Investigators:
Dr. Ronald E. Rolfe; Mr. Ronald Kuntzman

Cooperating Units: None

Man Years
Total: 1.5
Professional: .5
Other: 1.0

Patient Days: None

Project Description:

General Purpose of Research:

This laboratory has recently identified norepinephrine in brain by physico-chemical means and has shown that its distribution is like that of serotonin. It is obvious that two substances with such powerful physiologic activity must play some role in brain function.

Progress to date:

A number of years ago W. R. Hess stimulated many parts of the cat brain with implanted electrodes. From the resulting responses he concluded that two opposing systems in subcortical areas, coordinate autonomic, somatomotor and psychic functions. A major breakthrough in the over-all problem has been the realization that stimulation of the trophotropic system simulates actions of reserpine and chlorpromazine whereas stimulation of the ergotropic system simulates that of LSD and mescaline. The possibility is being explored that serotonin is the neurohormone of the trophotropic and norepinephrine of the ergotropic system.

Both amines satisfy minimal criteria for a central neurohormone: (1) They are present in a bound form in parts of brain where Hess found evidence for his systems; (2) monoamine oxidase, which inactivates both amines, is present in all parts of brain.

and is especially concentrated in the hypothalamus; (3) the parenteral injection of the amines produces central but opposite actions; (4) inhibition of monoamine oxidase exerts marked central action associated with an increase in the monoamines; and (5) LSD, which may antagonize normal function of serotonin and chlorpromazine that of norepinephrine, evoke marked central actions.

Considerable evidence has accrued in favor of the view that serotonin is the neurohormone of the trophotropic system. (Parenteral administration elicits sedation, ptosis, potentiation of hypnotics, depression of motor activity, electroencephalographic sleep-like patterns.) In contrast, the catechol amines may be the neurohormone of the ergotropic system since it produces in general the opposite effects.

A number of centrally acting drugs may now be classified according to their effects on the trophotropic or ergotropic systems as follows:

a. Drugs may produce the trophotropic syndrome by activation of the trophotropic system or by depression of the opposing ergotropic system

Evidence indicates that reserpine acts by stimulating the trophotropic system by causing the persistent release of serotonin while chlorpromazine acts by interfering with the action of brain norepinephrine.

b. A drug may produce the ergotropic syndrome by blocking the trophotropic system (thus unmasking the ergotropic) or by directly stimulating the ergotropic system

LSD has been considered to exert its effects by interfering with the action of serotonin at trophotropic synapses. A large number of congeners of norepinephrine that readily penetrate brain appear to stimulate the ergotropic system by simulating norepinephrine action.

This classification stresses pharmacologic actions of "mental" drugs rather than their behavioral effects. Since all ergotropic agents produce euphoria and psychotic behavior in sufficient doses, it is probable that this effect is part of the ergotropic syndrome.

Considering the viewpoint that autonomic, somatic and psychic functions of body are not static but are maintained in a balanced state by the two opposing systems, the various mental drugs act by upsetting this balance. They should be used therefore according to behavioral symptoms irrespective of the type of psychoses or neuroses.

One of the main difficulties in accepting serotonin as the hormone for the trophotropic system is its ergotropic effects when given in large amounts in the form of its precursor, 5-hydroxytryptophane (5HTP). Recent work indicates that the action of 5HTP is much more complicated than formerly considered and may involve the release of norepinephrine in brain. Furthermore, 5HTP, unlike other ergotropic agents, does not overcome the effects of reserpine and in fact can increase them.

LSD may not act centrally as a serotonin antagonist but seems to act, as does mescaline, etc., by simulating action of norepinephrine.

DOPA administered intravenously crosses into brain and is readily decarboxylated to yield DOPamine. This compound, just one hydroxyl from norepirepharine, yields typical ergotropic effects.

Direction of Current Research:

Studies will continue on whether LSD acts in brain by simulating norepinephrine, and bufotenine by blocking serotonin, and whether serotonin produces the effects typical of stimulation of the trophotropic system (reserpine-like effects).

Attempts to identify roles of norepinephrine and serotonin in brain will be made by investigating substances that block their formation, or differentially release them.

Incidental Findings of Significance: None

Part B included: Yes

Serial No. NMI-99

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards and Publications

Shore, Parkhurst A., Pletscher, Alfred, Tomich, Edward G., Carlsson, Arvid, Kuntzman, Renand, and Brodie, Bernard B. Role of Brain Serotonin in Reserpine Action. Ann. N. Y. Acad. Sci. 66: 609-615, 1957.

Brodie, Bernard B., and Shore, Parkhurst A. A Concept for a Role of Serotonin and Norepinephrine as Chemical Mediators in the Brain. Ann. N. Y. Acad. Sci. 66: 631-642, 1957.

Brodie, Bernard B., Bogdanski, Donald F., and Shore, Parkhurst A. Biochemical and Physiological Interpretation of the Action of Psychotropic Drugs. To be published as a chapter in a book entitled "Chemical Concepts of Psychoses." 1957.

Brodie, Bernard B. Serotonin and Norepinephrine Metabolism. Remarks at 25th Ross Pediatric Research Conference entitled "Consciousness and Chemical Environment in the Brain." In press, 1957.

Brodie, B. B., and Shore, P. A. Serotonin and Norepinephrine May Have Role in Central Autonomic Nervous System. Drug Trade News, 1957.

Brodie, Bernard B., and Shore, Parkhurst A. On a Role for Serotonin and Norepinephrine as Chemical Mediators in the Central autonomic Nervous System. Chapter in book entitled "Hormones, Brain Function and Behavior" by Academic Press, 1957.

Brodie, B. B. Storage and Release of 5-Hydroxytryptamine. Chapter in book on "Serotonin," Pergamon Press Ltd., London, 1957.

Brodie, Bernard B. Chapter in Macy Foundation Series on Neuropharmacology, Third Conference, 1957.

Honors and Awards: None

Form No. ORP-2
Oct. 1957

Serial No. NHI-100
1. Chemical Pharmacology
2. Clinical Pharmacology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the Mechanism of Action of Reserpine

Principal Investigator: Dr. Parkhurst A. Shore

Other Investigators: Mr. Ronald G. Kuntzman
Mrs. Jacqueline Olin

Cooperating Units: None

Man Years
Total: 1
Professional: .5
Other: .5

Patient Days: None

Project Description:

General Purpose of Research:

To show that the bewildering array of central effects elicited by reserpine results from stimulation of the trophotropic system, one of the two opposing systems indicated by W.R. Hess to integrate autonomic, somatomotor and psychic functions.

Progress to Date:

Previous work has indicated that reserpine and other active reserpine alkaloids have the singular property of impairing the mechanism that enables brain cells to store serotonin but without affecting the synthesis of the indole. As a result, serotonin leaves the cells in a continuous st. It has been postulated that the free serotonin stimulates synapses of the trophotropic thereby eliciting the observed effects.

We have now found that reserpine also impairs the capacity of body cells to bind norepinephrine in the adrenal medulla, peripheral nerve endings and in the brain. The norepinephrine depots in brain disappear rapidly at exactly the same rate as those of serotonin and the depletion of the two amines shows the same dosage-response curves. How the release of the two compounds is linked is not clear. The release of catechol amines from adrenal meduall occurs over a period of 16 hours and is due to a persistent stimulation of the adrenal nerve. The release of norepinephrine from the heart results from a direct action of the reserpine and occurs within 4 hours.

These findings have caused a re-evaluation of the mechanism of reserpine action. The loss of norepinephrine from peripheral nerve endings means that reserpine exerts a peripheral as well as a central effect, as shown by the experiments with reserpinized animals that indicate that sympathetic receptors no longer show a response to ganglionic stimulation.

Since reserpine impairs the capacity of brain cells to bind norepinephrine, then it is likely that this amine also continues to be formed and to leave the cells continuously. Reserpine may be pictured therefore as eliciting mixed effects by inducing a stimulation of both trophotropic and ergotropic synapses by serotonin and norepinephrine respectively with the net result that of trophotropic stimulation.

Trophotropic stimulation is indicated by the extreme miosis after reserpine which cannot be explained except by stimulation of its peripheral component, the parasympathetic system. On the other hand, the centrally mediated release of medullary amines is a reflection of persistent ergotropic stimulation since the adrenal medulla is innervated only by the sympathetic division of the autonomic nervous system.

A rather startling observation has been that the effects of DOPA (yields DOPamine in brain), amphetamine and LSD, which elicit effects opposite to those of reserpine, are actually potentiated by the Rauwolfia alkaloid. This finding, obviously important in the mechanism of reserpine action, is not understood.

Direction of Current Research:

Recent work in England indicates that serotonin is involved in the mechanism of intestinal peristalsis. It becomes important

therefore to ascertain whether serotonin release by reserpine is a direct one or is mediated through the central nervous system.

A new semi-synthetic Rauwolfia alkaloid from Ciba (3118) is said to elicit peripheral but not central effects. This compound may release norepinephrine and serotonin only from the peripheral depots. If so, it may prove a valuable drug in hypertension as well as a valuable tool in dissection of reserpine action.

Experiments will be undertaken to ascertain whether reserpine exerts both ergotropic and trophotropic results. These will involve studies of effects on denervated organs and the measurement of action potentials of sympathetic and parasympathetic peripheral nerves.

Incidental Findings of Significance: None

Part B included: Yes

Serial No. NHI-100

Part B: Honors, Awards and Publications

Shore, Parkhurst A., and Bernard B. Brodie. LSD-like Effects Elicited by Reserpine in Rabbits Pretreated with Iproniazid. Proc. of the Soc. for Exptl. Biol. and Med., 94: 433-435, 1957

Brodie, B.B., Tomich, E.G., Kuntzman, R., and Shore, P.A. On the Mechanism of Action of Reserpine: Effect of Reserpine on Capacity of Tissues to Bind Serotonin. J. of Pharm. and Expt. Therap. 119: 461-467, 1957

Brodie, Bernard B., Olin, Jacqueline S., Kuntzman, Ronald G., and Shore, Parkhurst A. Possible Interrelationship Between Release of Brain Norepinephrine and Serotonin by Reserpine. Science 125: 1293-1294, No. 3261, June 28, 1957.

Honors, Awards: None

Form No. ORP-2
Oct.1957

Serial No. NHI-101
1. Chemical Pharmacology
2. Clinical Pharmacology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on Short-Acting Synthetic Reserpine-like Drugs

Principal Investigator: Dr. Gertrude P. Quinn
Dr. Parkhurst A. Shore

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1/2
Professional: 1/2
Other: None

Patient Days: None

Project Description:

General Purpose of Research:

Until very recently, reserpine and the other tranquilizing Rauwolfia alkaloids have been the only substances known to cause the release of serotonin from the brain. There has now been synthesized in Switzerland a series of benzoquinolizine derivatives which cause many of the same pharmacologic effects as reserpine and which also cause a release of serotonin from the brains of animals. It is of considerable interest that the duration of effects of these compounds is very short, in contrast to reserpine which has a long duration of action. Furthermore, these compounds do not have an indole group in the molecule, which has been thought by some to be essential for serotonin release. With the use of this series of compounds it is expected that much of the theory of reserpine action via effects on serotonin and norepinephrine can be tested.

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Progress to Date:

This project was initiated in July 1957. It has already found, using one of the compounds, Ro 1-9569, that norepinephrine as well as serotonin is released from the brain, and that the duration of effects of the drug are related to the duration of effects on the brain amines. These results suggest that, as with reserpine, changes in these brain amines cause very pronounced central effects.

One marked difference between the action of the benzoquinolizines and reserpine is that reserpine acts by an "irreversible" mechanism; that is, the biochemical and pharmacologic effects persist long after the alkaloid has disappeared from the brain. It appears likely that the benzoquinolizines are "reversible" in that their effects are evident only as long as the drug is present.

Administration of Ro 1-9569 to rabbits a few minutes before reserpine blocks the long-lasting biochemical and pharmacologic effects of reserpine. Then animals are sedated for only a few hours, while if reserpine alone is administered, the animals are sedated for 2 or 3 days. This suggests that the Ro 1-9569 molecule occupies the sites on which reserpine ordinarily acts, and since reserpine rapidly leaves the tissues, it has little chance to act. This is also indicated by the observation that serotonin levels return to normal much more rapidly after the combination of drugs than after reserpine alone.

Of considerable interest are preliminary experiments which indicate that Ro 1-9569 may not release serotonin and norepinephrine from their peripheral depots. If verified, this would suggest that the sites in the brain which normally bind the amines may be different from the peripheral sites, or that perhaps Ro 1-9569 releases the brain amines by a different action even though it can block the biochemical effects of reserpine.

Direction of Current Research:

Further work is planned in comparing the actions of reserpine with those of the benzoquinolizines. It is expected that by virtue of the differential effects, some insight will be gained as to the relative importance of serotonin and norepinephrine in the action of these drugs.

As these compounds are short-acting, their use will supply information about the rates of biosynthesis of the two amines in the brain.

Incidental Findings of Significance: None

Part B included No /x/

Form No. ORP-2
Oct. 1957

Serial No. NHI-102
1. Chemical Pharmacology
2.
3. Bethesda, Md.

DHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on Chlorpromazine and Other Drugs that Interfere with the Action of Norepinephrine in Brain

Principal Investigator: Dr. Donald F. Bogdanski

Other Investigators: Mr. James Watts

Cooperating Units: None

Man Years:
Total: .50
Professional: .25
Other: .25

Patient Days: None

Project Description:

General Purpose of Research:

Chlorpromazine and a number of other synthetic "tranquilizing" agents elicit effects like those of reserpine, that is they act centrally to decrease sympathetic activity, lower skeletal muscle tone and induce sedation. The present studies indicate that chlorpromazine acts by a different mechanism than reserpine, that is it blocks one of the opposing systems in brain (the ergotropic) that integrate autonomic, skeletal muscle and psychic functions by interfering with the action of norepinephrine.

Progress to Date:

Chlorpromazine and its metabolite chlorpromazine sulfoxide appear to exert a predominantly central action.

A. Intraventricular injection of small amounts of chlorpromazine elicits sedation, miosis and relaxation of the nictitating membrane. The same amount of drug yielded no observable effects given parenterally and did not antagonize the effects of intravenous epinephrine.

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B. Parenteral chlorpromazine does not antagonize the effects of norepinephrine, the main peripheral sympathetic neurohormone. Chlorpromazine sulfoxide which exerts an action similar to chlorpromazine, blocks the effects of neither norepinephrine nor epinephrine.

C. The central effects of norepinephrine congeners including amphetamine, mescaline, tetrahydro-β-naphthylamine and DOPamine (from administered DOPA) are blocked by chlorpromazine.

The following data indicates that chlorpromazine acts by a different mechanism than reserpine.

A. In contrast to reserpine, chlorpromazine does not release serotonin or norepinephrine.

B. Rabbits treated with a monoamine oxidase inhibitor and then given reserpine which releases norepinephrine as well as serotonin, show excitement, psychomotor activity and an increased sympathetic activity. These effects are not antagonized by additional reserpine, but are antagonized by chlorpromazine.

C. DOPA administered to animals pretreated with reserpine enters brain and is decarboxylated to yield DOPamine. This substance reverses the action of reserpine. The resulting LSD-like effects are reversed by chlorpromazine, but not by reserpine.

D. Chlorpromazine unlike reserpine does not cause the release of adrenal catechol amines, it produces a potentiation of hypnotics that is not blocked by LSD, and it exerts a reversible action.

E. Chlorpromazine constricts the sympathetically denervated pupil much less than reserpine. In the dark the pupil dilates only slightly after reserpine, but to a considerable extent after chlorpromazine. This data suggests that chlorpromazine elicits miosis by central adrenergic blockade. Reserpine causes the pupillomotor center to be tonically active. Thus the pupillary effects of reserpine are due to parasympathetic stimulation.

Direction of Current Research:

These differences between chlorpromazine and reserpine are being studied in relation to their effects on physiologically active chemical agents in the brain. Thus, reserpine depletes

the brain of both norepinephrine and 5-hydroxytryptamine, whereas chlorpromazine affects neither. The chemical agents are being studied for possible relationships to the ergotropic and trophotropic systems of W.R. Hess. Centrally active adrenergic agents (blocked by chlorpromazine) produce effects similar to ergotropic stimulation, while reserpine "mimics" trophotropic stimulation. Current research is directed towards clarifying these postulated relationships.

A simple pharmacologic method for screening chlorpromazine-type tranquilizers will be tested in order to obviate the tedious behavioral screens.

Studies as to whether chlorpromazine blocks the depleting action of reserpine on medullary amines will be undertaken. Experiments as to whether it augments the central actions of reserpine will be carried out. The results of these studies may result in definitive proof as to whether chlorpromazine blocks the ergotropic system (central adrenergic blocking agent) and that reserpine stimulates the trophotrophic system.

Incidental Findings of Significance: None

Part B included No [X]

Form No. ORP-2
Oct. 1957

Serial No. NHI-103
1. Laboratory of Chemical Pharmacology
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the Mechanism of Action of Ergotropic Agents

Principal Investigator: Dr. Donald F. Bogdanski

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .5
Professional: .5
Other: 0

Patient Days: None

Project Description:

General Purpose of Research:

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.

Progress to Date:

Cocaine and LSD exert part of their effects by a central action. The increased blood pressure, tachycardia, hyperglycemia and mydriasis are reduced by spinal section or by the administration of hexamethonium. Excision of the superior cervical ganglion also prevents the mydriasis.

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The ergotropic agents are normally active, if not potentiated following reserpine, which releases the postulated ergotropic neurohumoral agent from the brain. However, chlorpromazine blocks the various actions of LSD, amphetamine, cocaine, tetrahydro-beta-naphthylamine and DOPA, a possible precursor of norepinephrine.

Direction of Current 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.

Incidental Findings of Significance: None

Part B included [X] No

Form No. ORP-2
Oct. 1957

Serial No. NMI-104
1. Chemical Pharmacology
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the Mechanism of Serotonin Binding in Tissues

Principal Investigator: Dr. F. Barbara Hughes
Other Investigators: Dr. Parkhurst A. Shore
None

Cooperating Units: None

Man Years:
Total: 1
Professional: 1
Other: None

Patient Days: None

Project Description:

General Purpose of Research:

Accumulating evidence points to an essential role for serotonin in brain function. The amine is said to be "bound" since it is stored in a form which is protected from the enzyme, monoamine oxidase. The question arises as to the nature of the binding and the mechanism whereby serotonin is released. The finding that the same Rauwolfia alkaloids that release serotonin from brain also release it from platelets suggests that the substances is held in both tissues by similar forces. Thus platelets may serve as an in vitro model for studying storage and release of serotonin.

Progress During Past Year:

Since serotonin is generally though to be complexed with an intracellular constituent, evidence for such a complex within platelets has been sought. It was found however, that the presence of such a complex could not be demonstrated for when the platelet membrane was ruptured, and the intracellular material dialyzed or subjected to ultrafiltration, it was found that the serotonin within the platelets acted as though it were in true solution. Furthermore, when placed in contact with a preparation of monoamine oxidase, the serotonin was destroyed at a rate which would indicate that all the serotonin was in an unbound state.

A possible explanation for the maintenance of serotonin

within platelets against a concentration gradient is that a transport mechanism acts toward this end. This possibility was tested by a comparison of the rates of uptake of serotonin and its synthetic dimethyl analogue, bufotenine. Both of these substances are extremely lipid insoluble, but serotonin is even more so than is bufotenine. It might be expected that if the uptake were by a non-specific means then the more lipid-soluble molecule (bufotenine) should be taken up to the greater degree. However, it was found that serotonin was taken up to a much greater extent than was bufotenine, indicating that a specific mechanism for the uptake of serotonin may exist. Consistent with this possibility is the observation that reserpine readily blocked the uptake of serotonin, but had no effect on bufotenine uptake. Additional evidence in favor of a transport mechanism is the finding that one molecule of reserpine releases hundreds of serotonin molecules from platelets.

Direction of Current Research:

Further experiments will be performed to test the possibility that a serotonin-transport mechanism exists. A possible role of adenosine triphosphate, which is present in platelets in high concentration, will be investigated. This substance could supply the energy needed for such a mechanism.

Binding of other amines by platelets will be investigated. We are especially interested in whether other normally occurring amines might be specifically taken up by platelets. For example, the uptake of norepinephrine and epinephrine will be compared with that of a synthetic catechol amine, butanefrine.

Incidental Findings of Significance: None

Part B included: Yes

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Part E: Honors, Awards and Publications

On the Nature of Serotonin Storage Possibility of a Transport Mechanism, Hughes, F.B., Shore, P.A., and Brodie, B.B., submitted for publication.

Honors and Awards: None

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Serial No. NHI-105
1. Chemical Pharmacology
2. Clinical Pharmacology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the Identification and Distribution of Catechol Amines in the Body

Principal Investigator: Dr. Parkhurst A. Shore

Other Investigators: Mr. Victor Cohn
Mrs. Jacqueline Olin

Cooperating Units: None

Man Years:
Total: 1
Professional: .5
Other: .5

Patient Days: None

Project Description:

General Purpose of Research:

Because of the considerable interest in possible functions of catechol amines in the brain, heart and other organs, there is need for a systematic examination of the distribution of catechol amines in the body and within various organs. To perform this study a sensitive technique is needed for the identification and assay of these substances in tissues.

Progress During Past Year:

A simple and sensitive chemical method has been perfected for the identification and assay of norepinephrine and epinephrine in brain and other organs. This method is based on extraction of the catechol amines from tissue homogenates and conversion to highly fluorescent products. Less than 1 microgram of catechol amines is needed for analysis and differentiation between norepinephrine and epinephrine. In rabbits, norepinephrine, the principal catechol amine in organs other than the adrenal gland, has been found to be distributed in brain in a non-uniform manner with the highest levels in the brain stem.

Surprisingly high levels of norepinephrine have been found in the heart, about 2 microgram per gm in rabbits and about 3 microgram per gm in dogs. Using tissue samples supplied by Dr. Harriet Maling of this laboratory, a careful study of the distribution of norepinephrine in dog heart is in progress in an attempt to discover its function in this organ. There is a fairly uniform distribution in the various parts of the heart with auricular tissue generally showing the highest concentration.

Direction of Current Research:

We are planning to compare the distribution of catechol amines in various species and to look for other catechol amines such as isoprenaline, which has been reported to be released by stimulation of the bronchial sympathetic nerves of cats.

Studies on the role of norepinephrine in the heart will continue. Utilizing the ability of reserpine to deplete the heart of norepinephrine, we plan to collaborate with other members of this laboratory on studies which show an altered behavior of heart tissue after disappearance of norepinephrine.

Incidental Findings of Significance: None

Part B included: Yes

Serial No. NHI-105

Part B: Honors, Awards, and Publications

Shore, P.A. and Olin, J.S. Identification and Chemical Assay of Norepinephrine in Brain and Other Tissues. J. Pharmacol. and Expt. Therap., in press.

Honors and Awards: None

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Serial No. NHI-106
1. Chemical Pharmacology
2. Clinical Pharmacology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on Catechol Amine Biosynthesis and Biotransformation

Principal Investigator: Dr. John Anthony Radford Mead
Dr. Parkhurst A. Shore
Other Investigators: Mr. Ronald Kuntzman

Cooperating Units: None

Man Years:
Total: 1
Professional: .5
Other: .5

Patient Days: None

Project Description:

General Purpose of Research:

Previous studies have lead to the suggestion that norepinephrine is a neurohumoral agent in the central autonomic nervous system. This suggestion has been based in part on the finding that the Rauwolfia group of tranquilizing agent releases norepinephrine as well as serotonin from the brain and other organs. Thus it has become of importance to learn more of the biosynthesis and metabolism of the catechol amines, especially norepinephrine.

Norepinephrine is believed to arise in the body from dihydroxyphenylalanine (DOPA) which is decarboxylated and then hydroxylated. Most of the studies on which this scheme is based have been carried out on peripheral organs. Whether a similar metabolic route exists in brain, or what part of the overall synthesis is performed in brain is to a large extent unknown.

The pathway of biotransformation of the catechol amines is controversial. The enzyme, monoamine oxidase, has been implicated, but since norepinephrine is a relatively poor substrate for this enzyme, the importance of monoamine oxidase in norepinephrine metabolism has been questioned.

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A recent observation that an enzyme system exists in the body which can methylate one of the phenolic hydroxyl groups suggests a possible alternate route of metabolism.

Progress During Past Year:

It has been demonstrated *in vitro* that norepinephrine is metabolized rather slowly by monoamine oxidase, about one-fifth the rate of serotonin. When brain norepinephrine and serotonin are released *in vivo* by administration of reserpine, the levels of the two amines decline at identical rates. As both the *in vitro* and *in vivo* metabolism can be almost completely blocked by known inhibitors of monoamine oxidase, it appears that this enzyme *in vivo* is highly active toward norepinephrine, and in fact is the enzyme of importance in normal metabolism of norepinephrine by the brain.

Although the presence of the methylating enzyme can be demonstrated in the brain, it appears from the experiments described above that this enzyme, if at all implicated in norepinephrine metabolism in brain, is of lesser importance. We have also demonstrated that a wide variety of centrally acting drugs do not affect the activity of the methylating enzyme, *in vitro*.

Experiments with possible precursors of norepinephrine have established that *in vivo*, DOPA, after intravenous injection can be rapidly decarboxylated in the brain to form dihydroxyphenylethylamine, whereas intravenous injection of another possible precursor, dihydroxyphenylserine, results in no rise in brain catechol amines. Whether this is because the brain decarboxylase enzyme is highly specific or whether only DOPA reaches the brain is under investigation.

Direction of Current Research:

Further investigation into the synthesis and biotransformation of norepinephrine will be carried out. It is planned to study the passage into the brain of possible precursors of norepinephrine, the specificity of brain DOPA decarboxylase, and the effects of inhibition of this enzyme.

We are also planning to study the turnover and size of the body pool of norepinephrine by measurement of a urinary excretion product in normal and reserpine treated animals. In doing this we hope to learn more of the metabolism whereby reserpine depletes the body of norepinephrine stores.

Incidental Findings of Significance: None

Serial No. NHI-106

Part B: Honors, Awards, and Publications:

On the Physiologic Significance of Monoamine Oxidase in Brain, Shore, P.A., Mead, J.A.R, Muntzman, R., Spector, S., and Brodie, B.B., Science, 126, No. 3282, 1063-1064, 1957.

Honors and Awards: None

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Oct. 1957

Serial No. NHI-107
1. Chemical Pharmacology
2. Clinical Pharmacology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the Mechanism of the Excitatory Action of Morphine in the Cat

Principal Investigator: Dr. Gertrude P. Quinn
Dr. P.A. Shore
Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1
Professional: 1
Other:

Patient Days: None

Project Description:

General Purpose of Research:

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 is excitatory in cats and which releases brain norepinephrine, is being compared with reserpine, which causes central depression and releases both serotonin and norepinephrine in brain.

Progress During the Past Year:

It has been found that the administration of morphine, 25 mg/kg, to cats causes considerable excitation and releases about two-thirds of the brain stem norepinephrine within six hours, behavior and norepinephrine levels returning to normal within 18 hours. There was no effect on brain serotonin levels.

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When reserpine (5 mg/kg) was administered to cats, there resulted a very marked sedation and a marked depletion (80 per cent) of both serotonin and norepinephrine from the brain. Depression and effects on the brain amines persisted for at least ten days.

It has been thought by some workers that the sedative action of reserpine might be explained simply on the basis of lowered brain levels of norepinephrine. The results of the morphine experiments cast doubt on this hypothesis since the studies show that a lowering of brain norepinephrine content does not necessarily cause sedation.

These experiments also supply some knowledge of the rate of biosynthesis of norepinephrine in the brain. Since brain norepinephrine returns to normal within 18 hours after morphine administration, it is evident that the prolonged depletion following reserpine administration is not a consequence of a normally slow turnover but must be due to a long lasting effect of the alkaloid.

Direction of Current Research:

Work is planned with a view to determining whether morphine causes its excitation through the action of released norepinephrine or whether the release is a secondary effect of a primary excitatory effect. It is also planned to examine the anatomical site of action of morphine excitation by use of appropriate surgical preparations.

Incidental Findings of Significance: None

Part B included: no

Form No. ORP-2
Oct. 1957

Serial No. NHI-108
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A

Project Title: Studies on the Physiologic and Biochemical Effects of Monoamine Oxidase Inhibitors

Principal Investigators: Dr. Sidney Spector; Dr. Parkhurst A. Shore

Other Investigators: Dr. Darwin Prockop

Cooperating Units: General Medicine Branch, NHI

Man Years
Total: 1.5
Professional: 1.5
Other: 0

Patient Days: None

Project Description:

General Purpose of Research:

Evidence has accumulated that iproniazid exerts a stimulatory effect on the central nervous system. Recent clinical reports have indicated that iproniazid is efficacious for depressed mental conditions.

Iproniazid has been reported to inhibit the enzyme, monoamine oxidase, which has been implicated in the metabolism of serotonin, norepinephrine and epinephrine. The present work was to ascertain the role of serotonin and norepinephrine in the stimulatory effects of monoamine oxidase inhibitors in the central nervous system.

Progress During Past Year:

a. The administration of a single large dose of iproniazid, 100 mg/kg s.c. to rabbits, elevated the brain level of serotonin threefold, and this level persisted for three days. The norepinephrine levels were elevated twofold, and likewise persisted for three days. During this period the animals exhibited no excitement.

b. Repeated administration of iproniazid, 25 mg/kg, s.c. produced excitement in three to four days at which time the sertonin level was again elevated two to threefold, while the norepinephrine was now also elevated two to threefold. The chronic administration of isoniazid, a chemical congener of iproniazid, lacking the isopropyl group, did not affect monoamine oxidase and did not produce excitement nor any alteration of the serotonin or norepinephrine levels.

c. Tissues other than the brain were analyzed. Only the blood levels of serotonin were found to be elevated during the chronic administration of iproniazid.

d. In work in collaboration with Dr. Albert Sjoerdsma and Dr. Louis Gillespie of the General Medicine Branch, two patients were placed on a daily regime of 3 mg/kg of iproniazid, and their blood serotonin levels followed. Within five days, their levels rose to three times their normal, at which time the drug was discontinued. Three weeks were required for the levels to return to normal range after the discontinuance of the drug.

Direction of Current Research:

Further studies on the brain monoamine oxidase system are planned. Other monoamine oxidase blocking agents more active than iproniazid will be investigated.

It is hoped that there will be uncovered a compound which will exert an immediate and reversible block of this enzyme. This would supply valuable information as to the normal role of the enzyme and the mechanism whereby the two brain amines function as neurohormones. Included with information which might be supplied would be a value for the rate of biosynthesis of serotonin and norepinephrine.

It appears that a great many chemical compounds in relatively high concentrations are blockers of monoamine oxidase. Studies will be carried out to determine whether the central toxic manifestations of overdoses of various drugs might result from a blockade in central monoamine oxidase activity.

Incidental Findings of Significance: None

Part B included: No

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Serial No. NHI-109
1. Chemical Pharmacology
2. Biochemistry of Drug Action
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

PART A

Project Title: Possible Implications of Serotonin and Norepinephrine in Convulsant and Anti-Convulsant Treatment

Principal Investigator: Dr. Parkhurst A. Shore

Other Investigators: Dr. Darwin Prockop

Cooperating Units: None

Man Years
Total: 0.5
Professional: 0.5
Other: 0

Patient Days: None

Project Description:

General Purpose of Research:

The action of some of the tranquilizing agents on brain serotonin and norepinephrine has led to a considerable effort to learn more of the function of these amines in the brain. A more recent finding (see report entitled "Studies on the Physiologic and Biochemical Effects of Monoamine Oxidase Inhibitors") is that iproniazid, a drug used as a "mood elevator" in depressed mental patients produces a rise in brain serotonin and norepinephrine.

For many years, convulsive therapy of various types has been used in treatment of psychotic patients. In an attempt to discover any possible role of serotonin or norepinephrine in convulsive therapy, we found in preliminary experiments that rabbits convulsing from one to three hours following insulin administration showed a rise in brain serotonin levels with a possible fall in norepinephrine levels.

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The changes in brain levels of these amines raise several questions concerning the mechanisms involved in convulsions. There are experiments showing that injected serotonin protects against convulsions. Also it has been found that reserpine, which lowers brain serotonin, also lowers the convulsive threshold. It is of interest that various anti-convulsant drugs have been reported as raising brain serotonin levels of rats.

Progress During Past Year:

This is a new project which started in August 1957. It has already been discovered that, as described above, insulin convulsions produce a twofold rise in brain serotonin and a fall in brain norepinephrine. It appears that the changes in amines are related to the convulsions and not to hypoglycemia since no change appears if convulsions are prevented by barbital. In a single experiment using metrazol as a convulsant, a rise in serotonin was observed.

Direction of Current Research:

The experiment with metrazol and insulin convulsions will be repeated. An instrument for administering electroshock convulsions is now being prepared. The effect of repeated convulsions on brain serotonin and norepinephrine will be observed. The threshold values for electroshock convulsions will be determined, and an attempt will be made to correlate changes in convulsive threshold with levels of brain serotonin and norepinephrine. The latter will be varied with combinations of reserpine, monoamine oxidase inhibitors such as iproniazid, and precursors of serotonin and norepinephrine. In the course of this, the monoamine oxidase inhibitors themselves will be tested as anti-convulsants.

Incidental Findings of Significance: None

Part B included: No

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Oct. 1957

Serial No. NHI-110
1. Chemical Pharmacology
2. Clinical Pharmacology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Function of Acetylcholine in the Central Nervous System

Principal Investigator: Dr. Alan Burkhalter and Dr. P.A. Shore

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.5
Professional: 0.5
Other: None

Patient Days: None

Project Description:

General Purpose of Research:

The study of the functions of proposed neurohumoral agents in the central nervous system is fundamental to the understanding of the central nervous system itself. Recent studies have indicated that serotonin and norepinephrine may act as neurohumoral agents in the central autonomic nervous system.

The central role of acetylcholine is as yet unproven. Although it is known that acetylcholine acts as a chemical transmitter in the peripheral nerves, there exists no conclusive evidence that acetylcholine has a similar function centrally. Consistent with this possibility, however, are the observations that acetylcholine applied directly to the cerebral cortex causes central stimulation, and also that cholinesterase inhibitors such as physostigmine exert a central action.

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Progress to Date:

This is a new project, initiated in October 1957.

Direction of Current Research:

In order to examine a possible central role of acetylcholine, a sensitive chemical method for the estimation of this substance in tissues is needed. Various techniques will be tried in a search for such a method.

Incidental Findings of Significance: None

Part B included: No

Form No. ORP-2
Oct. 1957

Serial No. MHI-111
1. Chemical Pharmacology
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Model Enzyme Systems in the Study of Drug Metabolism

Principal Investigator: Dr. James R. Gillette

Other Investigators: Mr. James V. Dingell

Cooperating Units: None

Man Years:
Total: 1.1
Professional: 0.1
Other: 1.0

Patient Days: None

Project Description:

General Purpose of Research:

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.

Progress During the Past Year:

As previously reported, a series of model systems have been shown to dealkylate the methyl derivatives of aniline and 4-aminoantipyrine. These systems functioned through peroxidase, oxidase and dehydrogenase mechanisms.

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Recent investigations have demonstrated that these model systems can carry out yet another microsomal reaction. The oxidation of chlorpromazine to its sulfoxide has been observed in both the methemoglobin and hematin peroxidase systems as well as the anaerobic ferric chloride system. An intermediate in this reaction has been prepared in the ferric chloride system and its properties are presently under investigation.

Direction of Current Research:

A system for non-specific aromatic hydroxylation, utilizing horseradish peroxidase and dihydroxy fumarate, has been described by Mason et al. (Mason, H.S., Onopryenko, I., and Buhler, D., Biochem. et Biophys. Acta 24, 225, 1957). The similarity of this system to the iron systems for dealkylation and sulfoxication would suggest that it may be capable of catalyzing both these and other of the microsomal reactions. Consequently, further studies will be devoted to determining the capabilities of this and other model systems.

Incidental Findings of Significance: None

Part B included: Yes

Serial No. NHI-111

Part B: Honors, Awards and Publications

Dingell, James V., The Dealkylation of Drugs and Other Foreign Compounds by Model Systems, Dissertation for the Degree of Master of Science, submitted to Georgetown University, 1957.

Gillette, James R., Dingell, James V., and Bernard B. Brodie, The dealkylation of N-alkylamines by Model Systems, submitted to Nature.

Honors, Awards: None

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Serial No. NHI-112
1. Chemical Pharmacology
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Drug Enzyme Systems (Sulfur Oxidation)

Principal Investigator: Dr. James R. Gillette

Other Investigators: Mr. Jerome J. Kamm

Cooperating Units: None

Man Years:
Total: 1.1
Professional: 0.1
Other: 1.0

Patient Days: None

Projected Description:

General Purpose of Research:

The study of the enzyme system responsible for the metabolism of chlorpromazine and other sulfur-containing compounds to the corresponding sulfoxides.

Progress During the Past Year:

Chlorpromazine is metabolized by TPNH dependent enzymes localized in the liver microsomes of the rabbit, dog, guinea pig, mouse and monkey. The sulfoxide was identified as the metabolite of chlorpromazine when guinea pig liver microsomes were used as the enzyme source. The disappearance of chlorpromazine requires oxygen since little if any disappearance is noted under anaerobic conditions.

Both chlorpromazine sulfoxide and chlorpromazine sulfone are also metabolized by TPNH and oxygen dependent enzymes localized in the liver microsomes of guinea pig. The rate of disappearance of the sulfoxide compound. Therefore, it is possible that the sulfone is an intermediate in the metabolism of the sulfoxide.

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Direction of Current Research:

An attempt will be made to identify the metabolite of chlorpromazine sulfoxide. Studies of the fate of chlorpromazine sulfone will be conducted. In addition several other compounds will be tested as substrates for the enzyme system responsible for chlorpromazine disappearance.

Incidental Findings of Significance:

When incubated with guinea pig liver homogenate under anaerobic conditions, chlorpromazine sulfoxide is reduced to chlorpromazine. Under the same conditions chlorpromazine sulfone is unaffected.

Part B included: Yes

Serial No. NHI-112

Part B: Honors, Awards and Publications:

Fouts, J.R., Kamm, J.J., and Brodie, B.B.: On the Reductive Detoxication of Prontosil Butter Yellow and Other Azo Compounds by Mammalian Liver Systems, J. Pharm. Exptl. Therap., 120, 291-300, 1957.

Honors, Awards: None

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Oct. 1957

Serial No. [illegible]
1. Chemical Pharmacology
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3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Metabolism of Toluene and Other Alkyl Hydrocarbon Sidechains by Mammalian Liver Preparations

Principal Investigator: Dr. James R. Gillette

Other Investigators: None

Cooperation Units: None

Man Years:
Total: 0.4
Professional: 0.4
Other: None

Patient Days: None

Project Description:

General Purpose of Research:

It has been known for many years that animals can oxidize toluene to benzoic acid. However, little is known of this biotransformation on an enzyme level. Since a number of foreign compounds can be metabolized by the microsomal fraction of liver, it is of interest to determine whether toluene is oxidized by the microsomal system. If this were the case, then the metabolism of a number of drugs might be understood.

Progress During the Past Year:

p-Nitrotoluene is metabolized to p-nitrobenzyl alcohol by a TPNH dependent enzyme system in rabbit liver microsomes. This enzyme system was not present in homogenates of kidney, lung, brain, skeletal muscle or heart. p-Nitrobenzyl alcohol is converted to p-nitrobenzoic acid by a DPN dependent enzyme system in the liver soluble fraction.

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Preliminary evidence indicates that the conversion of pentothal to pentothal carboxylic acid requires both the TPNH dependent enzyme system of liver microsomes and an enzyme system in the soluble fraction of liver. These data suggest that pentothal is converted to pentothal-w-alcohol by the microsomal system whilst the alcohol is converted to the acid by a soluble enzyme system.

Direction of Current Research:

Studies will be undertaken to determine whether other alkyl hydrocarbon sidechains are metabolized by the same mechanism as p-nitrotoluene and pentothal. It is possible that most if not all of the reactions involving the conversion of alkyl hydrocarbons to carboxylic acids require the action of both TPNH dependent microsomal systems to form primary alcohols and the action of the soluble fraction to convert the alcohols to carboxylic acids.

Incidental Findings of Significance: None

Part B included: No

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Oct. 1957

Serial No. NHI-114
1. Chemical Pharmacology
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Enzymatic Oxidation of Aromatic Alcohols and Aldehydes

Principal Investigator: Dr. James R. Gillette

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.4
Professional: 0.4
Other: None

Patient Days: None

Project Description:

General Purpose of Research:

It is well known that many aromatic primary alcohols and aldehydes are oxidized in vivo to their respective acids. However, very little is known about the metabolism of these compounds on an enzymatic level. Presumably the aromatic alcohols are oxidized to aromatic aldehydes and then to the carboxylic acids. The purpose of this investigation is to study the enzymes involved in these biotransformations. Such a study would help us to understand the metabolism of a number of drugs.

Progress During the Past Year:

p-Nitrobenzyl alcohol and p-nitrobenzaldehyde are oxidized to p-nitrobenzoic acid by DPN dependent enzyme systems in the soluble fraction of rabbit liver. p-Nitrobenzaldehyde can also be oxidized by a DPN dependent enzyme system of the kidney soluble fraction, while p-nitrobenzyl alcohol cannot be oxidized by this kidney fraction to any great extent. However, p-nitrobenzyl alcohol can be

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metabolized by a combination of purified liver alcohol dehydrogenase and the kidney soluble fraction. These findings are consistent with the view that p-nitrobenzyl alcohol is metabolized to p-nitrobenzaldehyde by an alcohol dehydrogenase and then to p-nitrobenzoic acid by an aldehyde dehydrogenase.

Direction of Current Research:

The metabolism of a number of other aromatic alcohols and aldehydes will be investigated.

Incidental Findings of Significance: None

Part B included: No

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Serial No. NHI-115
1. Chemical Pharmacology
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A

Project Title: Absorption of Drugs from the Human Stomach

Principal Investigator: Dr. Lewis S. Schanker

Other Investigators: Mr. Dominick J. Tocco

Cooperating Units: Laboratory of Kidney and Electrolyte Metabolism, NHI

Man Years:
Total: 0.7
Professional: 0.4
Other: 0.3

patient days: None

Project Description:

Progress During Past Year:

Previous work from this laboratory described the absorption of drugs by the rat somach and the secretion of parenterally administered drugs by the dog stomach. The results were consistent with the hypothesis that the gastric mucosa has the properties of a lipoid barrier, allowing rapid transfer of the unionized moieties of organic electrolytes while impeding the transfer of the ionized moieties. The present project, begun in January and completed in April 1957, was undertaken to extend the study of gastric absorption to the human.

Solutions of various drugs were administered via Levine tubes to fasted normal subjects in the left supine position. Incorporation of phenol red in the solutions permitted estimation of the initial and final volumes and the correction for the concentration change due to dilution. The decrease in concentration of drug was determined at 10 minute intervals for 40 minutes. Acidic drugs, such as aspirin and secobarbital, were rapidly absorbed. Moderately strong bases, such as quinine and ephedrine, were poorly absorbed, while the very weak base, antipyrine, was fairly rapidly absorbed. These results, which parallel the previous study of absorption by the rat stomach, demonstrate that the ionization constant and the acidic or basic nature of a drug are important in determining gastric absorption. The human stomach is potentially an important site of absorption for many orally administered

drugs if they are sufficiently soluble. Many drugs are absorbed from the human stomach more rapidly than ethyl alcohol, which heretofore was considered to be the unusual example of a drug which is absorbed by the stomach. Gastric absorption of drugs is both qualitatively and quantitatively similar in the human and rat.

Direction of Current Research:

Project completed in April 1957.

Incidental Findings of Significance:

None

Part B included: Yes

Serial No. NHI-115

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards and Publications

Publications:

Schanker, L. S., Shore, P. A., Brodie, B. B., and Hogben, C.A.M. Absorption of Drugs from the Stomach. I. The Rat. J. Pharmacol. and Expt. Therap. 120: 528-539, 1957.

Hogben, C.A.M., Schanker, L. S., Tocco, D. J., and Brodie, B. B. Absorption of Drugs from the Stomach. II. The Human. J. Pharmacol. and Expt. Therap. 120: 540-545, 1957.

Honors and Awards:

None

Form No. CRP-2
Oct. 1957

Serial No. NHI-116
1. Chemical Pharmacology, NHI
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A

Project Title: Absorption of Drugs by the Intestine

Principal Investigator: Dr. Lewis S. Schanker

Other Investigators: Mr. Dominick J. Tocco

Cooperating Units: Laboratory of Kidney and Electrolyte Metabolism, NHI

Man Years:
Total: 0.6
Professional: 0.3
Other: 0.3

Patient Days: None

Project Description:

Progress During the Past Year:

This work continues the study of the relationship between the intestinal absorption of drugs and their physico-chemical properties. In a previous phase of this study, when solutions of various drugs were passed through the entire rat small intestine in situ a single time, absorption rates of 0 to 60 per cent were observed. This technique revealed that absorption is a function of a drug's pKa, but it did not clearly distinguish relatively slow from very slow rates of absorption. Therefore the technique has been modified to magnify the differences between the rates of absorption of slowly and very slowly absorbed drugs.

The small intestine of the anesthetized rat was washed with a 1.0 mM drug solution containing a volume change indicator, phenol red or radioinulin. Then 30 ml. of fresh solution were continuously recirculated through the intestine for 3 hours. Total recoveries of phenol red and radioinulin were 98 $\pm$ 2 per cent. Drug absorption was calculated by correcting the concentration change of the drug by the concentration change of the volume change indicator. This technique clearly separated the absorption rates of drugs which are relatively slowly absorbed from those of drug which are very slowly absorbed.

The results support the previous finding that drugs are absorbed if they exist in solution in their unionized lipid-soluble form and that the intestinal mucosa is almost impermeable to the ionized form. Drugs like ephedrine and mecamylamine, which are highly ionized in the intestinal contents, were absorbed much more rapidly than quaternary ammonium bases and sulfonic acids which are completely ionized.

Direction of Current Research:

a. Ascertain whether lack of calcium ion in the intestinal lumen significantly modifies absorption.

b. Investigate the possible role of chelation in the absorption of certain drugs.

c. Investigate the effect of Diamox on absorption.

Incidental Findings of Significance: None

Part B included: Yes

Serial No. NHI-116

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

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 Expt. Therap. In press.

Form No. ORP-2
Oct. 1957

Serial No. NHI-117
1. Chemical Pharmacology
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Absorption of Drugs from the Colon

Principal Investigator: Dr. Lewis S. Schanker

Other Investigators: Mr. Panayotis A. Nafpliotis

Cooperating Units: None

Man Years:
Total: 1.4
Professional: 0.7
Other 0.7

Patient Days: None

Project Description:

Progress During Past Six Months:

This project was initiated May 1, 1957. Absorption of drugs from the colon was investigated in the anesthetized rat. A 1.0 mM solution of drug in isotonic saline (pH 7.2) at 37°C. was perfused through the large intestine at a rate of 0.2 ml. per minute. Absorption was determined by the decrease in drug concentration after a single passage through the colon. The rates of absorption of organic acids and bases are related to their ionization constants. Weak acids like thiopental and phenol with pKa's above 7 were rapidly absorbed; stronger acids like salicylic and benzoic with pKa's below 7 were absorbed less rapidly; very strong sulfonic acids were very slowly absorbed. A similar pattern was observed with basic drugs: the weak bases aniline and p-toluidine, with pKa's below 7, were rapidly absorbed; stronger bases like ephedrine and mecamylamine with pKa's above 7 were less rapidly absorbed; very strong quaternary ammonium bases were slowly absorbed. The results suggest that the mucosa of the large intestine like that of the small intestine has the properties of a lipoid barrier, preferentially allowing absorption of the lipid-soluble unionized form of an organic electrolyte.

Serial No. NHI-117

Direction of Current Research:

a. To investigate the influence of pH on the absorption of drugs from the colon.

b. To further characterize the mucosa of the colon by determining the steady-state distribution of drugs between the colonic lumen and blood.

Incidental Findings of Significance: None

Part B included: No

Form No. ORP-2
Oct. 1957

Serial No. NHI-118
1. Chemical Pharmacology
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Absorption of Natural Products from the Gastrointestinal Tract

Principal Investigator: Dr. Lewis S. Schanker

Other Investigators: Mr. Dominick J. Tocco

Cooperating Units: None

Man Years:
Total: 1.2
Professional: 0.6
Other: 0.6

Patient Days: None

Project Description:

Progress During Past Six Months:

This project, begun in May 1957, was undertaken to broaden our understanding of how a number of water-soluble substrates, especially the nucleic acid derivatives, cross biological membranes. Since this laboratory has designed a simple method of evaluating absorption from the intestine, the movement of a number of nucleic acid derivatives across the intestinal mucosa has been investigated.

Solutions of various purine and pyrimidine bases, nucleosides and nucleotides in isotonic saline (pH 7.2) were perfused through the entire small intestine of the anesthetized rat at a rate of 1.5 ml. per minute. The extent of absorption was determined by the difference in concentration of the solution entering the duodenum and leaving the ileum; this was expressed as the per cent absorption.

Serial No. NHI-118

The absorption of several of the nucleic acid derivatives studied was more rapid than might be expected from passive diffusion, considering their physico-chemical properties, especially their very low solubility in lipids. (Previous work from this laboratory revealed that rapid absorption of a drug occurred only if its undissociated form was lipid-soluble.) For two nucleosides, adenosine and inosine, it was found that the per cent absorption decreased when the concentration in the intestinal lumen was raised, suggesting active transport mechanisms for these compounds. It was further noted that a substantial proportion of adenosine is converted to inosine during the single rapid passage through the intestinal lumen.

Direction of Current Research:

a. To study the absorption of adenosine and inosine in greater detail to reveal the characteristics of their mechanisms of absorption.

b. To study other nucleic acid derivatives in more detail to ascertain the means by which they traverse the intestinal mucosa.

Part B included: No

Form No. OR-2
Oct. 1957

Serial No. NHI-119
1. Chemical Pharmacology, NHI
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A

Project Title: Entrance of Substances into the Central Nervous System

Principal Investigator: Dr. Lewis S. Schanker
Dr. Hermann Kurz

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.4
Professional: 0.4
Other:

Patient Days: None

Project Description:

General Purpose of Research:

To study the passage of both foreign and naturally occurring substances into the brain.

Progress During the Past Month:

This project was initiated in October 1957. The results of previous work from this laboratory on the passage of several drugs into the brain suggest that the barrier separating the brain from blood behaves towards foreign compounds as if it were lipoid in character. Other work from this laboratory dealing with the physiological disposition of barbiturates has shown that the entry of these drugs into the brain is related to their lipid solubilities. Procedures for more clearly characterizing the blood-brain barrier in the dog are now being developed.

Serial No. NHI-119

Direction of Current Research:

a. To design a simple procedure for comparing the rates of entry of a large number of drugs into the central nervous system of the dog.

b. To investigate the mechanisms involved in the passage of substances into the brain and to differentiate passive diffusion from active transport mechanisms.

Incidental Findings of Significance: None

Part B. Included: No

Form No. OH-2
Oct. 1957

Serial No. NHI-120
1. Chemical Pharmacology
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Secretion of Substances into Bile

Principal Investigator: Dr. Lewis S. Schanker

Other Investigators: Dr. Hermann Kurz

Cooperating Units: Laboratory of Kidney and Electrolyte Metabolism - NHI

Man Years:
Total: 0.6
Professional: 0.6
Other: 0

Patient Days: None

Project Description:

Progress During Past Six Months:

This project was initiated in April 1957. Prompted by earlier reports in the literature that inulin appears in canine bile, the transfer of inulin and sucrose between plasma and bile was re-examined. The renal pedicles of the anesthetized rat were ligated and the bile duct cannulated. Beginning 1 hour after the intravenous injection of the C^{14} labelled polysaccharide, three consecutive 90 minute samples of bile were collected. The radioactivity of these samples was compared to that of the terminal plasma sample. Plasma radioactivity was essentially stable during the collection of bile. The mean bile:plasma concentration ratios were 0.10 and 0.24 for inulin and sucrose respectively. These values suggest a significant porosity at some locus in the hepatobiliary system. Preliminary results with a third, smaller molecule, mannitol, indicate a bile: plasma concentration rationof about 1.10.

Direction of Current Research:

a. Determine the inulin, sucrose and mannitol "space" of the liver.

b. Study the biliary secretion of other substances, especially weak organic electrolytes.

Incidental Findings of Significance: None

Part B included: No.

Form No. ORP-2
Oct. 1957

Serial No. NHI-121
1. Laboratory of
Chemical Pharmacology, NHI
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A

Project Title: The Enzymatic Oxidation of Nicotine

Principal Investigator: Dr. Howard B. Hucker

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.00
Professional: 1.00
Other: none

Patient Days: None

Project Description:

General Purpose of Research:

Very little information is available concerning the enzymatic processes involved in the metabolism of nicotine, a drug of considerable pharmacological importance. Other workers have reported studies on the rates of excretion and metabolism of nicotine in the whole animal, as well as studies on its metabolism by tissue slices. The fate of nicotine in the animal organism has not yet been elucidated. It would be of interest, therefore, to examine the metabolism of nicotine at the cellular level in order to 1) localize the enzyme system responsible for its breakdown, 2) determine the requirements of the system, and 3) identify the metabolic products.

Progress During Past Year:

A method has been devised by which microgram quantities of nicotine may be accurately and rapidly assayed in various tissue preparations. The enzymatic breakdown of nicotine has been found to be catalyzed by liver microsomes and to require TPNH and oxygen. The reaction is inhibited by methylene blue, cytochrome c and β,β-diethylaminoethyl diphenyl propyl acetate (SKF 525-A). The inhibition induced by cytochrome c is reversed by addition of cyanide.

The possibility that demethylation of nicotine to nornicotine is a major metabolic pathway was eliminated, since no formaldehyde was detected in the incubation mixture.

Direction of Current Research:

Studies are now in progress on the nature of the possible metabolites formed from nicotine by incubation in the system mentioned. The acid, γ-(3-pyridyl)-γ-methylaminobutyric acid, which would result from oxidative ruptute of the pyrrolidine ring of nicotine, is at present being investigated. Other likely compounds will be studied also in an effort to explain the metabolic fate of nicotine.

Incidental Findings of Significance: None

Part B included: No

Form No. ORP-2
Oct. 1957

Serial No. NHI-122
1. Chemical Pharmacology
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Fate of Reserpine in the Body

Principal Investigator: Dr. Sidney M. Hess
Dr. J. Rieder

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.4
Professional: 1.4
Other:

Patient Days: None

Project Description:

General Purpose of Research:

The importance of reserpine in clinical practice, and more important its use as a tool in the study of the central autonomic nervous system, makes it imperative that information concerning its fate in the body be known.

Progress During Past Year:

Guinea pig liver homogenate is able to hydrolyze reserpine in vitro, while rabbit liver slices or homogenate is unable to do so. Since the drug is degraded in vitro by both these animals a careful examination was made of the means by which these species handle the drug.

Using whole animal homogenates, guinea pigs were found to contain 81 per cent of reserpine plus methyl reserpate one hour after receiving the drug, 5 mg./kg. Four hours after a similar injection the content of reserpine, plus methyl reserpate had dropped to 54 per cent of the injected dose. No evidence for

glucuronide conjugation was found to account for the disappearance of reserpine.

There was some evidence that the level of methyl reserpate in the plasma of guinea pigs receiving reserpine was higher than the level in the plasma of rabbits similarly treated. Methyl reserpate could be detected in the plasma of guinea pigs 72 hours after an injection of reserpine or of methyl reserpate.

No evidence was found for the presence of reserpic acid in the urine of rabbits or guinea pigs after an injection of reserpine.

Rabbit received	Per cent dose recovered in urine 24 hours after injection			
	Reserpine	Methyl reserpate	Reserpic acid	Total
Reserpine	2%	11%	0%	13%
Methyl reserpate		16%	13%	29%
Reserpic acid			49%	49%

Rabbits and guinea pigs received reserpine labeled in the carboxyl group of the trimethoxybenzoic acid moiety. The urine collected during the first 24 hours after the injection contained one-fourth to one-half of the total activity injected. The radioactive material in the urine was largely trimethoxybenzoic acid, and an occasional trace of reserpine.

To compare the excretion of trimethoxybenzoic acid after injection in rabbits and guinea pigs, the compound was administered and the urine collected. The activity in the urine in each case accounted for 80-100 per cent of the administered dose. About half was recovered as unchanged TMBA and the other half was recovered as conjugated TMBA.

From this data it has been concluded that although there is a large difference in the ability of rabbit and guinea pig livers to metabolize reserpine *in vitro*, the difference *in vivo* is not quite as striking.

Serial No. NMI-123

To find the remaining 50 per cent of the activity as yet unaccounted for in the rabbit, the intestinal contents were extracted and about 13 per cent of the total dose was recovered.

About 7 per cent of the administered radioactive dose of reserpine is metabolized to $C^{14}O_2$ in 24 hours.

Direction of Current Research:

Studies on the metabolism of reserpine in mammals are continuing. Efforts will be made to find the 30-40 per cent of the radioactivity as yet unaccountable. There is as yet no clue for the changes occurring in rings A through E of the reserpine molecule and investigations on this aspect are being made.

Incidental Findings of Significance: None

Part B included: No

Form No. ORP-2
Oct. 1957

Serial No. NHI-124
1. Chemical Pharmacology, NHI
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A

Project Title: Studies in Evolution:
I. Studies on the Distribution of Mammalian Microsomal Enzymes in Other Species

Principal Investigator: Mr. Roger P. Maickel

Other Investigators: Dr. Werner R. Jondorf

Cooperating Units: None

Man Years:
Total: 0.4
Professional: 0.4
Other: 0

Patient Days: None

Project Description:

General Purpose of Research:

There are many instances in the evolutionary scale in which nature was faced with a problem which had to be solved if development was 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 excrete lipid soluble compounds suggests that with the escape from water, there also developed special systems for making fat-soluble substances which are 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 gaining insight on the development of enzymes responsible for the biochemistry of function.

Progress During the Past Year:

a. A brief survey of the distribution of several of the "microsomal" enzyme systems indicated that, in general, mammalian

species have the systems for hydroxylation of aromatic rings, and cleavage of ether linkages. This distribution was similar to that previously found for dealkylation and sidechain oxidation activity. A study of various reptiles indicated that they possess at least some of the microsomal systems.

b. When representative compounds of six microsomal pathways of drug metabolism were administered to frogs, intraperitoneally, 70-90 per cent was excreted unchanged through the skin of the animal in 18-24 hours. Analysis of the frogs at the same time gave a total recovery of 90-105 per cent of the administered dose, which would indicate that there is no metabolism of these compounds in the whole animal. In vitro studies of the microsomes showed no ability to oxidize foreign compounds. Similar results were obtained in fish.

c. Preliminary studies indicated that there is no nitro-reductase activity in either frogs or fish.

Direction of Current Research:

Other enzyme systems for the metabolism of drugs and other foreign organic compounds will be studied to see which of them are special mechanisms for the disposal of foreign compounds.

Incidental Findings of Significance: None

Part B included: No

Form No. ORP-2
Oct. 1957

Serial No. NHI-125
1. Laboratory of Chemical Pharmacology, NHI
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A

Project Title: Studies in Evolution
II. Conjugation and Excretion of Phenols by the Fish and Amphibia

Principal Investigator: Mr. Roger P. Maickel

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.4
Professional: 0.4
Other: 0

Patient Days: None

Project Description:

General Purpose of Research:

a. Administration of phenols (such as phenolphthalein, 8-hydroxyquinoline, alpha-naphthol and para-nitrophenol) to frogs by intraperitoneal injection by stomach tube, or merely by placing the animal in a solution of the compound so that it would permeate the animals through the skin, resulted in excretion of 60 - 75 per cent of the phenol in a conjugated form in 18 - 24 hours and virtually complete conjugation and excretion (90 - 95 per cent) in 48 hours.

b. If the animals were maintained in a glucose free medium for 72 - 96 hours before administration of the phenol, their glucuronic acid supply would be decreased, and unless they were given access to a supply of glucose after the phenol was administered it would be conjugated primarily as the ethereal sulfate (sulfate/glucuronide = 5/1). However, if the aqueous environment in which the animals were kept after administration of the phenol were made 1mM in glucose, the phenols were excreted primarily as the glucuronide (glucuronide/sulfate = 4/1).

c. In frog tadpoles no conjugation of phenols as either glucuronide or sulfate could be found after intraperitoneal administration or after placing the animals in a solution of the phenol for 18 - 24 hours.

d. Fish were killed by very small amounts of phenols (as little as 10 ugms. of p-nitrophenol) administered by intraperitoneal injection. They were also much more sensitive to phenols present in the environment than were frogs. In no instances could any indication of glucuronides or ethereal sulfates be found, nor could the recovery of administered phenols (normally 85 - 94 per cent(be increased by hydrolytic treatments which would liberate conjugated phenols.

Direction of Current Research:

a. Attempts will be made to determine what part of the glucuronide and sulfate conjugating mechanisms are missing in the fish and tadpole, that is whether the glucuronyl transfer enzyme is present and whether UDPG is a common constituent.

b. A wider variety of animals will be studied to give assurance of the fact that both of these results are characteristic of both salt and fresh water fish, and of the various species of frogs.

c. Attempts will be made to associate appearance of conjugative mechanisms with some other event that would explain their development and the possibility that bilirubin is formed in frogs, but not in fish and that a conjugation mechanism was necessary to excrete this compound will be examined.

Incidental Findings of Significance:

It is possible to deplete the frog of the ability to form glucuronides by depleting the glucose supply. However, it is not possible to deplete the animal of the sulfate conjugating ability.

Part B included: No

Form No. ORP-2
Oct. 1957

Serial No. NHI-126
1. Chemical Pharmacology
NHI
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A

Project Title: Studies in Evolution
III. Metabolism of Various Drugs and Foreign Compounds by the Toad (Bufo marinus)

Principal Investigator: Mr. Roger P. Maickel

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.3
Professional: 0.3
Other: 0

Patient Days: None

Project Description:

General Purpose of Research:

The toad, though an amphibian, is a land dweller and therefore lacks the damp, semi-permeable skin of the frog and salamander. Since it cannot excrete appreciable quantities of lipid-soluble compounds through the kidney, it must dispose of non-polar foreign compounds by converting them into less lipid-soluble forms which can then be excreted through the kidney. Previous studies indicate that ordinary amphibia lack the microsomal oxidative systems common to mammals. Thus, the problem of oxidation of drugs must have been solved independently in this amphibian off-shoot. The important question that arises is whether a function which is solved more than once in evolution is solved differently each time.

Progress During the Past Year:

a. The toad metabolized a number of drugs after parenteral administration to the extent of about 70 per cent of the administered dose in 18 - 24 hours. These drugs were those which are oxidized by various pathways in mammalian microsomes.

b. However, *in vitro* studies with the same series of compounds did not produce similar results. Only aminopyrine, antipyrine and chlorpromazine are metabolized by toad liver homogenate. None of the metabolites reported in mammals could be found as a result of the metabolism by the toad. Toad microsomes plus TPNH did not oxidize drugs, nor did the toad have any TPNH oxidase requirement similar to that of mammals.

c. Oxygen is required for the toad system, and studies with various substituted pyrazolones indicate that the pyrazolone ring is broken. SKF 525-A has no effect on the enzymes, either *in vivo* or *in vitro*, while cyanide acts as an inhibitor in the metabolism by homogenate or slices.

d. An indication that the mechanisms of the toad are different than those in mammals comes from the fact that the enzyme activity is located in the 144,000 x g supernatant liquid fraction rather than in the microsomes.

Direction of Current Research:

a. An intensive study of the mechanism of drug oxidation in the toad will be made. Special emphasis will be placed upon the isolation and identification of metabolites, as a prelude to an elucidation of the mechanisms involved.

b. Studies will be made to determine why some compounds disappear *in vivo* but not *in vitro*.

Incidental Findings of Significance: None

Part B included: No

Form No. ORP-2
Oct. 1957

Serial No. NHI-127
1. Chemical Pharmacology, NHI
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A

Project Title: Studies in Evolution
IV. The Metabolism of Drugs by Arthropods

Principal Investigator: Dr. Werner R. Jondorf

Other Investigators: None

Cooperating Units: None

Man Years
Total: 0.4
Professional: 0.4
Other: 0

Patient Days: None

Project Description:

General Purpose of Research:

In connection with the concept of biochemical evolution, it is of interest to determine where those enzyme systems responsible for the metabolism of drugs in vivo first appeared. These enzyme systems accomplish a variety of basic reactions such as dealkylation, deamination, hydroxylation, ether cleavage and sulfoxide formation. Since it is known that the higher terrestrial animals can metabolize drugs and other foreign compounds by mobilizing one or more of these enzymatic defense mechanisms, it was natural that animals lower in the evolutionary scale should be studied from the same standpoint. Eventually such a study would lead to the compilation of data relating to significant biochemical differentiation of species.

Progress During Past Six Months:

a. Insects

In experiments with house crickets (Acheta domestica L.) the compounds were injected into the thorax. After 1-3 hours the insects were homogenized and the drug remaining was estimated spectrophotometrically.

The cricket rapidly metabolized aniline, N-methyl aniline, acetanilide and amphetamine. Chlorpromazine, pyramidon and p-methoxyacetanilide were metabolized to a lesser extent.

b. Crayfish

Freshwater crayfish were injected with drugs, dorsally between the carapace and first segment of the abdomen. After 24 hours, surprisingly, the crayfish was found to have metabolized the drugs, though much less readily than crickets. Thus 38 per cent of the administered thiopental was metabolized, while chlorpromazine, amphetamine and pyramidon were metabolized to the extent of only about 20 per cent.

None of the drugs was excreted, indicating that the gills of the crayfish, unlike those of fish, did not have a lipoidal barrier. This would explain why it was necessary for crayfish and presumably other crustacea to have developed mechanisms for the metabolism of lipid soluble foreign compounds.

Direction of Current Research:

a. Although the metabolism of foreign compounds by insects has been the subject of numerous studies in relation to the metabolism of insecticides, little is known of the details and mechanisms of these metabolic changes. This proposed to elaborate on the pathways of metabolism in insects.

b. It is proposed to examine metabolic changes in crayfish at the cellular level.

c. The range of studies is being extended to other species of arthropods including a marine species and a terrestrial species with an aquatic larval stage.

Interim Findings of Significance:

When crayfish were immersed in solutions of drugs they did not exhibit any of the expected pharmacological symptoms. Their behavior was normal, even when the concentration of drug (thiopental) was stepped up to 1 gram per liter. This contrasted quite markedly with the behavior of goldfish similarly immersed. The goldfish died within 1/2 hour, whereas the crayfish were unaffected even after 24 hours. Analyses of the solutions at the end of the experiments revealed that none of the drug had been metabolized. This again suggests that the crayfish possesses very special gills and absorption apparatus.

Part B included: No

Form No. ORP-2
Oct. 1957

Serial No. NHI-128
1. Chemical Pharmacology, NHI
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A

Project Title: A Study of Prolonged States of Exaggerated Drug-Induced Cardiac Arrhythmias in Dogs and Their Relation to Fatty Changes in the Myocardium

Principal Investigator: Dr. Harriet M. Maling

Other Investigators: Mrs. Martha A. Williams
Dr. Benjamin Highman

Cooperating Units: National Institute of Arthritis and Metabolic Diseases

Man Years:
Total: 1.2
Professional: 0.6
Other: 0.6

Patient Days: None

Project Description:

General Purpose of Research:

This project is a study of the physiological and biochemical mechanisms underlying prolonged states of exaggerated cardiac arrhythmias in dogs. An understanding of these mechanisms may provide a rational basis for the selection of potential new antiarrhythmic drugs.

Progress During the Past Year:

Two methods have been established 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. (2) A similar state of exaggerated drug-induced cardiac arrhythmias lasting 2 to 5 days occurs in dogs following an intravenous infusion of a large dose of norepinephrine (0.51 to 0.96 mgm. per kgm.). During this time, test doses of

either epinephrine (1.0 and 10.3 microgm. per kgm.) or norepinephrine (0.95 and 9.5 microgm. per kgm.) induce ventricular tachycardia. These exaggerated ectopic responses occurred after norepinephrine infusions of 2.1 microgm. per kgm. per min. for 4 hours as well as after infusion of 6.2 to 11.5 microgm. per kgm. per min. for 80 min., or after a total dose of 0.85 mgm. per kgm. divided into 5 or 6 injections 20 minutes apart. Similar exaggerated responses to 9.5 microgm. per kgm. norepinephrine occurred in 3 of 6 dogs after infusion of 0.55 mgm. per kgm. epinephrine and in the 2 survivors of 9 dogs infused with 0.92 mgm. per kgm. epinephrine.

Fatty changes are prominent in the hearts of dogs sacrificed the day after infusions of large doses of either epinephrine or norepinephrine. The severity of these fatty changes seems to be roughly correlated with the severity of the exaggerated arrhythmias. It therefore seems plausible that an altered metabolism of myocardial fibers involving fat underlies these abnormal states of exaggerated arrhythmias. This hypothesis gains support from the fact that myocardial infarcts in dogs are surrounded by a region of marked fatty changes for a number of days after coronary occlusion.

Serum cholesterol levels were elevated 8, 14, and 44 per cent in 3 dogs the day after infusion of 0.51 mgm. per kgm. norepinephrine. In 2 dogs, the serum cholesterol rose from 90 and 123 mgm. per cent to 151 and 327 mgm. per cent the day after administration of 0.85 mgm. per kgm. norepinephrine in divided doses. Preliminary studies indicate that the elevation in serum cholesterol may persist for several days. This suggests that catechol amines may play a role in the genesis of atherosclerosis.

Infusions of catechol amines also cause a transient hyperglycemia. In 9 dogs, the blood glucose rose from a mean value of 117 per cent to 142 mgm. per cent at the end of an infusion of 0.51 mgm. per kgm. norepinephrine. The hyperglycemia is much more striking with equimolar infusions of epinephrine. In 6 dogs, the blood glucose rose from a mean of 119 mgm. per cent to 275 mgm. per cent at the end of an infusion of 0.55 mgm. per kgm. epinephrine. The hyperglycemia produced by epinephrine and norepinephrine infusions is markedly potentiated by pretreatment with cortisone. One dog weighing 13 kgm., pretreated with 100 mgm. cortisone subcutaneously daily for 6 days, showed a rise in blood glucose from 110 mgm. per cent before infusion to 400 mgm. per cent at the end of an infusion of 0.9 mgm. per kgm. norepinephrine. Three normal untreated dogs given similar infusions of norepinephrine had blood glucose levels of 136, 144, and 148 per cent at the end of infusion.

Since carbohydrate and lipid metabolism are closely interrelated, these hyperglycemic effects accompanying infusions of catechol amines support the assumption that metabolic effects are at least partially responsible for the fatty changes observed in several organs. Additional evidence for the importance of metabolic effects of catechol amines is a trend toward an elevated serum alkaline phosphatase the day after these infusions. However, generalized vasoconstriction and myocardial hypoxia resulting from excessive stimulation of the heart during norepinephrine infusions may be responsible for the fatty changes rather than specific metabolic actions of catechol amines.

In 2 dogs it has been shown that the adrenergic blocking agent, dibenzyline, in a dose of 2 mgm. per kgm. given 1 hour before an infusion of 0.85 mgm. per kgm. norepinephrine (1 dog) or an equimolar infusion of epinephrine (0.92 mgm. per kgm.) prevents both the fatty changes in the myocardium and the exaggerated arrhythmias (1 dog). Dibenzyline does not prevent the hyperglycemic effects of catechol amines. The prevention of the exaggerated arrhythmias by dibenzyline is not a direct antiarrhythmic action of dibenzyline since this drug in doses up to 8 mgm. per kgm. given the day after an infusion of 0.51 mgm. per kgm. norepinephrine does not block the exaggerated arrhythmias induced by test doses of norepinephrine. Dibenzyline does block the pressor effects accompanying these infusions and probably an appreciable amount of the direct myocardial stimulation. One interpretation of these experiments with dibenzyline is that relative hypoxia of the heart during infusions of catechol amines is prevented by the adrenergic blockade, thus removing the cause of the fatty changes. An alternative interpretation is that dibenzyline may block specific effects of catechol amines upon lipid metabolism, even though the hyperglycemic effects are not blocked.

Direction of Current Research:

Experiments are in progress to determine whether fatty changes in the myocardium and exaggerated cardiac arrhythmias are causally related. It is now being determined whether the duration of fatty changes in the myocardium following catechol amine infusions in normal dogs and following coronary artery occlusion corresponds to the duration of exaggerated arrhythmias.

Research into other possible methods of producing fatty changes in the myocardium may answer the questions of whether exaggerated arrhythmias invariably occur when fatty changes are present and whether fatty changes are invariably present when arrhythmias are exaggerated. It is planned to study the effects of: (1) exposure to high altitudes, (2) infusions of other myocardial stimulants such as serotonin and isoproterenol. The infusions with the vasodepressor cardiac stimulant isoproterenol

should clarify the roles of myocardial stimulation and increased peripheral resistance, (3) infusions of methoxamine resulting in a sustained rise in blood pressure without myocardial stimulation, and (4) administration of chlorinated hydrocarbons.

The causes of the fatty changes are still unknown. The experiments with exposure to high altitudes and with infusions of other agents should help to clarify the role of hypoxia in these abnormal states.

A study will be made of the fat and carbohydrate of the heart both in normal dogs and in dogs during abnormal states of exaggerated arrhythmias Changes in total fat and in the lipid portion of the heart will be followed. The fatty changes which have been demonstrated histologically suggest an increase in neutral fat.

The effects of lipotropic agents such as BAL, methionine, cysteine, and glucose will be studied with respect to their ability to prevent fatty changes in the heart and abnormal states of exaggerated arrhythmias The influence of various diets will be determined, including high fat diets, high cholesterol diets, high carbohydrate diets, and high protein diets. Histological changes in arteries and various organs including the liver, kidney, and brain will be followed, in addition to changes in the heart.

The role of norepinephrine in lipid metabolism will be studied. Blood cholesterol levels will be followed. Blood phospholipids will be determined. The effects of pretreatment with cortisone before norepinephrine infusions will be followed.

Incidental Findings of Significance: None

Part B included: Yes

Serial No. NHI-128

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications:

Maling, Harriet M., and Neil C. Moran. Ventricular Arrhythmias Induced by Sympathomimetic Amines in Unanesthetized Dogs Following Coronary Artery Occlusion. Circulation Research, 5: 409-413, 1957.

Maling, Harriet M., and Benjamin Highman. Delayed Effects Upon the Heart of Large Doses of Epinephrine and Norepinephrine in Dogs. Presented at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics, Baltimore, Md., September 6, 1957.

Form No. ORP-2
Oct. 1957

Serial No. NHI-129
1. Chemical Pharmacology
2. Physiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Ability of Dogs with Myocardial Infarcts to Withstand Exposure to High Altitudes

Principal Investigator: Dr. Harriet M. Maling

Other Investigators: Mrs. Martha A. Williams

Cooperating Units: In collaboration with Dr. Benjamin Highman and Mr. Milton Parker, National Institutes of Arthritis and Metabolic Disease

Man Years:
Total: 0.7
Professional: 0.3
Other: 0.4

Project Description:

General Purpose of Research:

This project was undertaken to determine the ability of dogs with myocardial infarcts to survive exposure to high altitudes.

Progress During the Past Year:

Dogs were placed in a high altitude chamber and the chamber evacuated to the equivalent of 34,000 feet. The exposure to 34,000 feet was continued for 3 hours. Electrocardiograms were recorded at frequent intervals during the exposure. The dogs were observed during the exposure through a window in the chamber.

Dogs with myocardial infarcts 3 or more days after coronary occlusion (ligation of the anterior descending coronary artery) survived exposure to 34,000 feet, as well as control dogs. The mortality results are summarized in Table I.

Serial No. NHI-129

Table I. Mortality of Dogs Exposed to 34,000 feet for Three Hours

Type of Dog	Number of Dogs		
	Died	Survived	Total
Normal	1	9	10
Sham-operated, 2 days after surgery	3	3	6
With myocardial infarct, 2 days after coronary ligation	5	1	6
With myocardial infarct, 3 days after coronary ligation	0	4	4
With myocardial infarct, 5 to 14 days after ligation	0	4	4

Autopsies were performed on all dogs which died during exposure. At least one dog with a myocardial infarct died from ventricular fibrillation, as indicated by electrocardiographic tracings. Sham-operated dogs which died during exposure apparently died from respiratory failure.

Survivors were sacrificed the day after exposure and autopsied. The histological studies have not been finished.

Direction of Current Research:

The analysis of the electrocardiographic tracings and the histological studies will be completed.

Incidental Findings of Significance: None

Part B included: No

Form No. ORP-2
Oct. 1957

Serial No. NHI-130
1. Chemical Pharmacology
2. Physiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the Pharmacology of Inhibitors of Monoamine Oxidase

Principal Investigator: Dr. Harriet M. Maling

Other Investigator: Mr. William M. Butler, Jr.

Cooperating Units: In collaboration with Dr. Benjamin Highman of the National Institute of Arthritis and Metabolic Diseases.

Man Years:
Total: 0.2
Professional: 0.1
Other: 0.1

Patient Days: None

Project Description:

General Purpose of Research:

Monoamine oxidase has been shown to play an important role in the metabolism of both norepinephrine and serotonin. This project is an investigation of the pharmacology of Marsilid and other monoamine oxidase inhibitors which may have important therapeutic value in treatment of hypertension and mental illness.

Progress During Past Month:

In 4 dogs, daily subcutaneous injections of 25 mg/kg Marsilid base for 6 to 8 days caused a gradual fall in mean arterial pressure, reflecting primarily a fall in diastolic pressure. Postural hypotension was observed during tilting as early as the fourth day. The pressor response to the ganglionic stimulant tetramethylammonium bromide (TMA) was abolished. Walking became difficult. The dogs were unsteady when standing. The hind legs showed a tendency to sink towards the floor. The urine became dark brown. The dogs lost their appetite and lost weight.

The general depression just described may be the result of the profound anemia produced by Marsilid in dogs. The hematocrit fell as low as 9 in 1 dog after 6 daily doses of 25 mg/kg Marsilid base. The serum became colored and icteric indices of 6 and 9 units were observed in 2 dogs after 4 daily doses of 25 mg/kg Marsilid base.

Serum cholesterol levels tend to rise in dogs given daily subcutaneous injections of Marsilid. In 2 dogs the serum cholesterol rose from 94 and 143 before treatment to 195 and 184 mg. per cent after 6 daily doses of Marsilid.

Dogs were sacrificed after 6 to 8 daily doses of Marsilid and autopsied. The bile was dark brown. The viscera seemed pale, probably because of the anemia. Histological studies are in progress.

Direction of Current Research:

Prolonged experiments on the effects of daily administration of Marsilid in dogs will be continued. Observations will also be made on cats, rabbits, rats, and mice. The experiments will be extended to other monoamine oxidase inhibitors. A possible influence of monoamine oxidase inhibitors upon lipid metabolism will be followed because of effects of norepinephrine as described in another report.

Incidental Findings of Significance: None

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 1958

Total:	$470,574
Direct:	$274,879
Reimbursements:	195,695

Serial No. NI-[illegible]

1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Factors Involved in the Production of Ammonia by the Kidney, and the Relation of Production to Ammonia Excretion in the Dog.

Principal Investigator: Jack Orloff

Other Investigator: Floyd Rector

Man Years:
Total: 3
Professional: 2
Other: 1

Patient Days: None

Project Description:

Factors that alter ammonia production both acutely and chronically are being studied. It has been shown that ammonia excretion varies acutely with changes in urine pH. By comparing ammonia production, estimated from the renal A-V difference in dogs with indwelling catheters in the renal vein, and ammonia excretion as urine pH is acutely altered by infusions of acidifying and alkalinizing salts, it will be possible to assess the relative importance of acute changes in ammonia production versus changes in the distribution of ammonia between cells, blood and urine in the regulation of ammonia excretion. Further, these studies should permit an evaluation of the extent to which ammonia production and ammonia utilization by cellular metabolic processes are regulated by the intracellular concentration of ammonia.

Chronically acidotic dogs excrete more ammonia than do normal dogs. It has been shown by others that most of this urinary ammonia during acidosis is derived from the hydrolysis of glutamine. In acidotic rats and guinea pigs this adaptive increase in ammonia excretion can be partially accounted for by an increased glutaminase I activity in renal slices and homogenates. As previously reported no such enzymatic change is noted in homogneates of dog kidney. Therefore the ability of intact kidney slices to hydrolyze glutamine, as well as the other enzyme systems that are known to catalyze the hydrolysis of glutamine (pyruvate-activated glutaminase II and glutamine synthetase) will be examined for adaptive changes during acidosis and alkalosis.

Progress During the Past six months:

A. The technique for renal vein catheterization and a reliable method for measuring blood ammonia have been perfected.

B. Measurements have shown that as urine pH is acutely shifted from the acid to the alkaline range ammonia excretion decreases logarithmically, renal A-V ammonia increases 2-3 fold and net production of ammonia decreases slightly. A redistribution of ammonia from urine to blood, rather than the fall in production, appears to be the major reason for the fall in ammonia excretion. An elevation of the intracellular concentration of ammonia might be the cause for the fall in net ammonia production, but since in all of the studies renal blood flow fell progressively, a deterioration in renal function cannot be excluded as the cause of the diminished production. Attempts are now being made to sustain a constant renal blood flow by maintaining blood pressure and blood volume constant with whole blood transfusions

C. Methods for assaying the hydrolysis of glutamine by intact kidney slices and by pyruvate-activated glutaminase II in homogenates have been established. Glutamine synthetase, which is easily measured in rat kidney, cannot be detected in dog kidney by any of three different methods.

Direction of Current Research:

Mechanisms for the regulation of ammonia production other than adaptive changes in the glutamine hydrolyzing enzyme systems are bein explored. Possibilities being considered are intracellular inhibitors or activators, and substrate accumulation by an active transport process.

Part B Included: No.

Form No. ORP-2
Oct. 1957

Serial No [illegible]-132
1. Kidney & Electrolyte Metaboli
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A:

Project Title: The Effect of Potassium on Urinary Dilution

Principal Investigator: Jack Orloff

Other Investigators: Henry N. Wagner, Jr.
Douglas G. Davidson

Man Years:
Total: 4
Professional: 3
Other: 1

Patient Days: None

Project Description:

The initial step in the elaboration of a hypotonic urine is the reabsorption of sodium and attendant anion from isosmotic precursor urine in an area of limited permeability to water. Part and possibly all Na^+ reabsorption is effected by exchange for H^+ and K^+ ions. Secretion of H^+ ions in exchange for Na^+ with a secondary mechanism for anion removal will decrease the concentration of osmotically active substances in urine, forming solute-free H_2O (C_{H_2O}). On the other hand secretion of K^+ in exchange for Na^+ will not alter urine osmolality. Furthermore, the inhibitory effect of K^+ on H^+ secretion and consequent decrease in Na^+ reabsorption, were this to occur at the at the diluting site, should interfere with the production of C_{H_2O}. This hypothesis was tested in female dogs undergoing maximal water diuresis.

Water diuresis was induced in all dogs. 1000 micromoles/min. of NaCl was infused during 2-3 control periods, followed by the gradual substitution of equiosmolar amounts of KCl. In all studies C_{H_2O} diminished in association with a rise in plasma potassium and an increase in potassium excretion. In contrast the enhancement of potassium excretion following diamox was associated with a fall in C_{H_2O} only if initial water diuresis was induced by the administration of hypotonic Na HCO_3. Although it was not possible to exclude a stimulatory effect of the elevated plasma potassium on ADH secretion, the results are consistent with a direct renal effect of potassium as outlined above. The enhanced secretion of potassium and the interference with hydrogen ion secretion may limit the extent of urinary dilution.

An attempt to rule out an effect of the rise in plasma potassium on ADH secretion will be made by introducing KCl directly into one renal artery of a dog and comparing the change in C_{H_2O} on the experimental side with the contralateral kidney serving as a control. It has not been possible to prepare dogs with permanent diabetes insipidus to further test the hypothesis.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Publication

An abstract was submitted to May 1957 meeting of the Society for Clinical Investigation.

Form No. ORP-2
Oct. 1957

Serial No. NHI-133
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A:

Project Title: Effect of Strophanthidine on Renal Function in the Chicken

Principal Investigator: Jack Orloff

Other Investigator: Maurice Burg

Man Years:
Total: 3
Professional: 2
Other: 1

Patient Days: None

Project Description:

These experiments were designed to investigate the effect of strophanthidine on electrolyte excretion. The unique circulation of the kidney in the chicken has been discussed in previous reports. If the cardiac glycoside has a specific effect on the renal tubule, it should be theoretically possible to demonstrate a unilateral change in electrolyte excretion when the substance is injected into one leg of the chicken.

1. Effects of strophanthidine:

a. Strophanthidine in doses of 500 micrograms per minute dissolved in 5% propylene glycol or 8% ethanol was infused in one leg vein of chickens during the course of mannitol or water diuresis. There was an immediate unilateral increase of urine flow, sodium excretion, and urine pH. Potassium excretion fell when it had been high initially due to potassium sulfate administration. Potassium excretion was constant or rose slightly when it was initially low. The rise in potassium excretion under these circumstances was less than at comparable levels of sodium excretion following sodium sulfate administration. These results are interpreted as being due to an inhibition of renal electrolyte transport mechanisms by strophanthidine.

b. Effect of H strophanthidine.

H-strophanthidine is a biologically inactive derivative of strophanthidine, obtained by saturation of the lactone ring. It had no effect on renal electrolyte excretion in identical dosage and in the same organic solvent. This establishes the specificity of the observed effect.

Direction of Current Research:

Investigation of the effect of strophanthidine on the excretion of radioactive potassium in an attempt to further elucidate the mechanism of potassium excretion.

Part B Included: Yes

Serial No. NHI-133

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Publication

Paper presented before October meeting of Washington Chapter of American Federation for Clinical Research.

Form No. ORP-2
Oct 1957

Serial No. NME-134
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Renal Action of Parathyroid Extract in the Chicken

Principal Investigator: Norman G. Levinsky

Other Investigators: Douglas G. Davidson
Robert W. Berliner

Man Years;
Total: 4
Professional: 3
Other: 1

Patient Days: None

Project Description:

The renal action of parathyroid extract has been studied in the chicken, in which a renal portal system facilitates separation of renal tubular from systemic and glomerular effects. It was found that net secretion of phosphate occurs in the chicken kidney both at endogenous plasma phosphate levels and during the infusion of phosphate. The rate of secretion seems to be independent of the plasma phosphate concentration. Infusion of parathyroid extract into one renal portal system results in a unilateral increase in phosphate excretion. In some experiments unilateral net secretion of phosphate occurred. No consistent changes in glomerular filtration rate or plasma phosphate concentration were noted. These results indicate that parathyroid extract has a direct action on the renal tubule in the chicken.

This project has been completed.

Part B Included: yes

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Publication:

Levinsky, N. G. and D. G. Davidson. Renal Action of Parathyroid Extract in the Chicken. Amer. J. Physiol. 191:530 , 1957.

Serial No. [illegible]-135
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Maintenance of Potassium Excretion Despite Reduction of Glomerular Filtration During Sodium Diuresis

Principal Investigator: Douglas G. Davidson

Other Investigators: Norman G. Levinsky
Robert W. Berliner

Man Years:
Total: 4
Professional: 3
Other: 1

Patient Days: None

Project Description:

The excretion of potassium was studied in the dog during reduction of the glomerular filtration rate by means of an inflatable cuff placed around one renal artery. In experiments in which no attempt was made to maintain high rates of sodium excretion, potassium excretion fell with the decrease in glomerular filtration rate. When high rates of sodium excretion were maintained by the administration of mercurials, sodium sulfate, or Diamox, the rate of potassium excretion was maintained in spite of reductions of the glomerular filtration rate by up to 40 percent. This was true both at high and low rates of potassium excretion. The results support the interpretation that reabsorption of filtered potassium is essentially complete and that the urinary potassium is derived from secreted potassium. The dependence of potassium excretion on sodium excretion provides evidence that the secretion of potassium occurs by an exchange of potassium for sodium in the distal tubule.

Part B Included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Publication

Davidson, D. G., Levinsky, N.G. and Berliner, R.W. Maintenance of Potassium Excretion Despite Reduction of Glomerular Filtration During Sodium Diuresis. J. Clin. Investigation (In Press)

Form No. CRP-2
Oct. 1957

Serial No. [illegible]
1. Kidney & Electrolyte Metabolism
2. National Heart Institute
3. Bldg. 10, Room 6N316

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title:

Studies on Diuretics

Principal Investigator: T. J. Kennedy, Jr.

Other Investigators:

Cooperating Units:

Man Years
Total: 4
Professional: 1
Other: 3

Patient Days: 901

Project Description:

Fourteen patients have been admitted for study of the diuretic potency of chlorothiazide in congestive heart failure within the last year. In addition the response to this drug of one patient with the nephrotic syndrome was observed. The general plan of study was to allow the patients to stabilize on a roughly constant diet of low sodium content, and on a more or less constant level of daily activity. When weight had become normal, sodium chloride and diuretic were introduced simultaneously into the regimen, in an effort to evaluate the capacity of the subjects to handle the increased sodium chloride load. During the course of the studies, the volume as well as the electrolyte composition of the 24 hour urine was measured. In some of the patients the idealized protocol could not be followed, due to the critical condition of the patients.

At present time, several conclusions seem warranted. 1) Many patients with so-called severe congestive heart failure will, on long term hospitalization and restriction of activity not only reach a satisfactory state of compensation on a diet restricted in sodium, but will also evince a capacity to excrete salt loads of significant orders of magnitude. One of the patients could maintain sodium chloride balance and remained free of edema even when challenged with 15 gm/day of sodium chloride. These patients were not included in the study. 2) A second group of patients could be selected who were characterized by some inability to excrete salt loads, retaining significant amounts of the loads, but always excreting 20-80% of the intake. In these patients, chlorothiazide in doses of from 1-4 gram/day was capable of inducing a zero sodium balance, and in the case of edematous subjects, a

negative balance until the edema had disappeared. This group comprised about 1/2 of the cases studied. No severe disturbances in potassium balance was observed in the group.

3) A third group of patients showed a remarkable inability to excrete salt. These patients excreted less than 1 mEq./day of sodium and gained weight slowly on a daily sodium intake of 10 mEq, and where given larger salt loads again demonstrated virtually complete retention and a rate of weight (edema) increase predictable on the basis of the electrolyte balance. This group constituted roughly 50% of the cases studied. In this group, chlorothiazide exhibition did not modify except transiently and insignificantly the positive sodium balance at levels of dietary sodium intake of either 10 or 60 mEq./day. However, exhibition of the drug led to an increase in the rate of excretion of potassium in all of these patients and in several, negative potassium balance persisted inspite of supplementation of dietary intake by up to 100 mEq. KCl/day. The potassium loss led to moderate to severe hypokalemic alkalosis. The interpretation placed on these findings is that chlorothiazide inhibited proximal tubular potassium transport, increased the load of counter ion (Na) to the Na-K exchange site where an overly active exchange mechanism resulted in virtually complete reabsorption of Na with equivalent extrusion of K.

Part B included: No.

Form No. ORP-2
Oct. 1957

Serial No. NM-13-7
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Analysis, Distribution and Exchangeability of Chloride in Tissues

Principal Investigator: Ernest Cotlove, M.D.

Man Years:
Total: 2
Professional: 1
Other: 1

Patient Days: None

Project Dezcription:

The determination of accurate values for chloride in tissues is an essential preliminary to a study of chloride distribution and exchangeability. Chemical analysis of chloride in tissues is complicated by the presence of interfering substances, and by potential formation of volatile chloride products. Different methods of preparation and analysis of tissue have yielded markedly different values. In the present study chloride has been measured chemically by the automatic electrometric titration procedure described in an accompanying report and by radioassy using added Cl-35. Results to date have shown that some preparative procedures give erroneously high values due to silver-combining groups other than chloride, and one commonly used procedure gives erroneously low values due to loss of chloride. Several procedures examined give accurate values as confirmed by radioassy controls.

Direction of Current Research:

Additional measurements will be made in other tissues. The exchangeability of chloride in tissues will be examined by the use of radioactive Cl-36. A comparison will be made of the distribution of radioactive C-14 inulin and sucrose with that of non-radioactive chloride.

Part B Included: No.

Form No. ORP-2
Oct. 1957

Serial No. NMI-136
1. Kidney & Electrolyte
Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Progress Report
Calendar Year 1957

Part A.

Project Title: The Effect of Pitressin and Solute Diuresis on Solute-Free Water Excretion

Principal Investigator: Jack Orloff

Other Investigators: Henry N. Wagner, Jr.
Douglas G. Davidson

Man Years:
Total: 4
Professional: 3
Other: 1

Patient Days: None

Project Description:

This has been discussed in the previous report. In essence a quantitative evaluation of the simultaneous effect of graded doses of vasopressin and variations in solute excretion on the clearance of solute-free water was attempted. The independent effect of alterations in glomerular filtration rate was minimized insofar as possible.

In addition to the results noted in the previous report, i.e. that vasopressin and solute diuresis exert antagonistic effects on the excretion of free water, it has been noted that persistent urine hypotonicity is produced when massive solute diuresis is effected during the administration of presumably maximal doses of vasopressin. It has not been possible to promote the elaboration of a hypertonic urine despite addition of as much as 150 mUnits/Kg/min of vasopressin. Whether this is due to a change in the responsiveness of the tubule membrane to vasopressin or represents merely the effect of massive solute excretion on free-water clearance has not been determined.

In association with Dr. Norman Levinsky the effect of massive solute diuresis on the response to vasopressin is being reinvestigated. It is hoped to determine whether membrane permeability on the concentrating mechanism is altered in the course of these studies or whether the back-diffusion of free-water is so limited by the osmotic diuretic as to effect the excretion of a hypotonic urine despite a maximal effect of vasopressin on the permeability of the tubule membrane to water.

Incidental Findings of Significance:

The administration of large water loads to prehydrated dogs has resulted in the elaboration of a hypotonic urine despite administration of 50 mU/Kg/hr. of vasopressin in 3 out of 6 studies. This "unresponsiveness" to vasopressin is

being investigated in collaboration with Drs. Maurice Burg and Norman Levinsky. It is presumed that the marked fall in plasma osmolality noted in the successful studies may either alter membrane permeability or electrolyte reabsorption in the diluting area.

Part B: Publications

Orloff, Jack, H.N.Wagner, Jr., and D.G.Davidson. The Effect of Vasopressin and Solute Excretion on the Excretion of Solute-Free Water. J. of Clinical Investigation (In Press) 1958

Form No. ORP-2
Oct. 1957

Serial No. MH-139
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Mechanism for Concentration of the Urine

Principal Investigator: Robert W. Berliner

Other Investigators: Norman G. Levinsky
Douglas G. Davidson
Murray Eden

Cooperating Unit: Laboratory of Technical Development.

Man Years:
Total: 5
Professional: 4
Other: 1

Patient Days: None

Project Description:

A hypothesis which describes the mechanism by which the kidney concentrates the urine has been formulated and experiments have been directed towards obtaining new data to bear on the validity of various parts of the theory. According to the new formulation, the active transport of sodium (chloride) by the water-impermeable loop of Henle creates an hypertonic interstitial fluid in the medulla of the kidney. The glomerular filtrate is isosmotically reduced in volume in the proximal tubule and, when antidiuretic hormone is present, further reduced to a small volume of isosmotic fluid in the distal tubule. The small volume of fluid then enters the water-permeable collecting ducts, which are intermixed with the loops of Henle in the medulla. Water is then moved along the concentration gradient from the isotonic tubular fluid to the hypertonic interstitial fluid, resulting in a concentrated urine. The collecting ducts are also permeable to urea, which diffuses from the tubular fluid to the extracellular fluid of the medulla. There urea, like the sodium transported by the loop of Henle, is trapped in high concentration by virtue of a countercurrent flow of blood to the medulla.

The presence of high concentrations of sodium in the medulla of the dog has been confirmed by direct analysis of medullary tissue under various conditions of urine concentration. During marked acute reduction of the glomerular filtration rate, it was noted that the concentration of maximally concentrated urine tended to fall. Experiments have been done which show a relation between the decrease in urine concentration and low concentrations of urea (and sometimes sodium) in the medulla.

Direction of Current Research:

Further experiments along the above lines are planned. In addition, attempts will be made to measure the sodium concentration of medullary interstitial fluid obtained directly by micropuncture techniques.

Part B Included: No.

Oct. 1957

Serial No. [illegible]
1. Kidney & Electrolyte Metabolism
2.
3.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Acidification of the Urine

Principal Investigator: T. J. Kennedy, Jr.

Other Investigators: R. W. Berliner
Norman Levinsky
Murray Eden

Cooperating Unit:

Man Years:
Total: 6
Professional: 4
Other: 2

Patient Days:

Project Description:

The purpose is to gain further insight into the detailed mechanism for urinary acidification. The specific facet under investigation at the present moment has to do with the problem of why the CO_2 tension of urine and plasma may be disparate.

A kinetic formulation of reattainment of equilibrium after the addition of acid to buffered and unbuffered solutions of bicarbonate has been completed. The equations derived have been shown to closely describe the actually observed experimental results. By use of equations and the high speed computing techniques (available through the National Bureau of Standards) as well as by actual experimental observations, it has been possible to evaluate quantitatively the influence on reaction kinetics of various parameters such as buffer concentration, buffer pKa, buffer catalytic coefficient, etc.

An attempt has been made to reinterpret some of the in vivo experimental observations in the light of this kinetic knowledge, on the assumption that CO_2 diffusion equilibrium is maintained throughout the course of the nephron, and that subsequently, CO_2 tension disparaties appear when the reaction delayed in reaching equilibrium by buffer, goes to completion in a CO_2 impermeable area.

More recently, in the light of work by others in this laboratory on the role of the renal medullary circulation as a trapping device for maintaining high concentrations of sodium and urea in the inner zone of the medulla, the possibility of CO_2 trapping was entertained. Experiments are in progress to determine whether or not such does in fact occur, and if so, what influence it exerts on the CO_2 tension of urine.

Part B included: No.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the Function of Single Nephrons in Necturus Maculosus

Principal Investigator: T. J. Kennedy, Jr.

Man Years:
Total: 1
Professional: 1
Other: None

Patient Days: None

Project Description:

The study of the operaton performed by the nephron in the formation of urine. The objectives of this program are to study in as intimate detail as possible the mechanisms whereby the nephron modifies glomerular filtrate into the final urine. Classical clearance techniques permit the evaluation of the chemical composition of both the initial material (glomerular filtrate) presented to the nephron as well as the final material, urine, elaborated by the nephron. However, there is no method evaluating what happens along the course of the nephron other than a direct approach to the various segments of this structure.

Considerable effort has been expended in an attempt to develop methods for Na and K on micro samples. Details are covered in a separate progress report by Dr. R. L. Bowman, and Mr. Whitehouse.

Direction of Current Research:

1. Development of micro flame photometer, micro freezing point depression instrument.

2. Measurement of EMF's across tubular membrane after exhibition of compounds known to modify electrolyte transport.

Part B included: No

Serial No. [redacted]
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Isolation and Characterization of a Cardiac-Active Protein System in Serum

Principal Investigator: Stephen Hajdu

Other Investigator: Edward Leonard

Cooperating Unit: Laboratory of Cardiovascular Physiology, H-5-341.

Man Years:
Total: 2
Professional: 2

Patient Days: 150

Project Description:

Initial investigations (thermolability, inactivation by organic solvents, retention by collodion membranes during ultrafiltration) suggested the protein nature of the system, and it was further revealed that more than one component was involved. One component (referred to as component L) binds strongly to the frog heart used for assay, and is the factor the concentration of which is elevated in hypertensive plasmas. The action of the system on the isolated frog heart is to restore the capacity to develop tension, to eliminate the staircase effect, and in the presence of a high concentration of component L to cause contracture. These actions can be reversed by washing the heart with several changes of physiological salt solution. The washing does not remove component L, but does remove a washable factor needed for activity of the system. Preliminary isolation procedures were designed to separate component L from the washable factor. The sensitivity of the proteins dictated the avoidance of organic solvents for separation; sodium sulfate is the most satisfactory precipitating agent.

Component L is now prepared from a serum containing the factor in high concentration, hypertensive human or normal dog serum being satisfactory. The protein is precipitated by dialysis of serum against 25% sodium sulfate, loss of the washable factor activity being assured by prior dilution of the serum. L activity survives in 50% glycerol at -20 degrees centigrade for many weeks.

Component L, when added to the heart, binds strongly and cannot be washed out over a period of many hours. A heart with component L bound is referred to as "sensitized". A sensitized heart with a large amount of component L bound serves as the assay system for the washable factor, addition of which then causes contracture.

During the attempted isolation of the washable activity, it was found that great losses occurred if room temperature dialysis was carried out against large volumes of calcium-free solutions. These losses can be prevented if calcium is present in all precipitating solutions. The washable fraction activity can be recovered practically without loss by precipitating the proteins of normal serum

all the serum globulins and a small fraction of the total albumin. (Albumin is not needed for the activity). Further purification of the washable activity yielded 2 protein fractions, neither of which alone has any effect on a sensitized heart, but which together reproduce the original activity. One fraction is precipitated from 0.17% sodium sulfate; the other, from 22-30%. The washable activity, them, is comprised of 2 protein fractions.

A number of experiments suggest that calcium plays an important role in the action of the washable fraction. Under the appropriate conditions, serum which is dialyzed against calcium-free solutions at 25 degrees centigrade loses washable activity. The loss can be prevented if dialysis is performed at 4 degrees, or if 5mM/L calcium chloride is present in the dialysis solution. Samples which have lost activity on dialysis can sometimes be reactivated by the addition of calcium. Free, ionic calcium does not of course substitute for washable activity. These results suggest that calcium may be bound to one of the proteins that comprise the washable fraction. Supporting evidence is found in the fact that this system can cause contracture of the sensitized heart in the absence of ionic calcium; and no other substance or system is known to induce heart muscle contraction in a calcium-free medium.

Direction of current research:

An attempt will be made to demonstrate more directly that this system binds calcium and to investigate the conditions governing its release. The physiological action of the system in tissues other than the frog heart will also be studied.

PHS- NIH
Individual Project Report
Calendar Year 1957

Part B: Publications:

Hajdu, S., Edward Leonard, R.P.Akers, Action of Human Plasma on the Isolated Frog Heart. Circulation Research. 5:319-322, 1957.

Hajdu, S. Bioassay for Cardiac Active Principles Based on the Staircase Phenomenon of the Frog Heart. J. of Pharm. and Exptl. Therap. 120:90-98,1957.

Hajdu, S., H. Weiss and E. Titus. The Isolation of a Cardiac Active Principle from Mammalian Tissue. J. of Pharm. and Exptl. Therap. 120:99-113,1957.

Form No. ORM-2
Oct. 1957

Serial No. NHE -9
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Progress Report
Calendar Year 1957

Part A.

Project Title: Salt Transfer across the Small Intestinal Epithelium

Principal Investigator: Dr. C. Adrian M. Hogben

Other Investigator: Dr. David Chalfin, Postdoctoral fellowship, PHS

Man Years:
Total: 2
Professional: 2

Patient Days: None

Project Description:

The pattern of Na and Cl flux across the intestinal epithelium is one of almost constant Na movement and a higher duodenal Cl flux and lower ileal Cl flux. The very high conductance of intestinal epithelium precluded concluding whether Na alone, Cl alone or both are actively transported during saline absorption.

Jejunal transfer in the presence of HCO_3^- was studied in greater detail. A net movement of Cl was demonstrated and the absorption of saline from jejunal sacs was associated with a fall in the concentration of Cl within the lumen.

Exposing the mucosal surface of the jejunem to 1/4 strength saline is not associated with a significant potential but if the same solution is made isotonic by the addition of sucrose a 20 mV potential difference (mucosa positive) appears. If the mucosal solution is kept constant there is a transmembrane potential which is roughly proportional to the log of the NaCl concentration difference. The obvious qualitative changes are an increase in the serosal to mucosal Cl flux when hypotonic and a reduction in the same flux when the solution is isotonic but deficient in NaCl. These observations while not yet completely analyzed are sufficiently unique that they may provide a new basis for studying the interrelationship between salt and water movement across the intestinal epithelium.

Part B Included: No

Form No. ORP-2
Oct. 1957

Serial No. NHI-144
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Progress Report
Calendar Year 1957

Part A.

Project Title: The Transfer of Salt and Water across the Dog Large Intestine In Vivo

Principal Investigator: Dr. I. Cooperstein

Other Investigator: Dr. S. Brockman

Cooperating Unit: Laboratory of Cardiovascular Physiology.

Man Years: Patient Days: None
Total: 2
Professional: 2

Project Description:

A blind loop of the dog's large intestine has been created with an ileal-rectal anastomosis to maintain the intestinal continuity.

The mammalian large intestine has been reported to have a sodium-potassium exchange and chloride bicarbonate exchange mechanism. The sodium and chloride moves from lumen to plasma. Previous studies have not taken the electrical gradient into account. By using a volume indicator and radioactive ions while recording the transmembrane potential - further clarification of electrolyte transport in large intestine can be obtained. Project started Oct. 1957 to July 1958.

Part B included: No

Form 96 ORP-2
Oct. 1957

Serial No. [illegible]
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Progress Report
Calendar Year 1957

Part A.

Project Title: Salt and Water Transfer across the Isolated Bullfrog Large Intestine

Principal Investigator: Dr. Irving Cooperstein

Other Investigator: Dr. C.A.M.Hogben

Man Years:
Total: 2
Professional: 2

Patient Days: None

Project Description:

The movement of Na^{22} and Cl^{36} has been measured across the isolated large intestine of the frog. This tissue is characterized by a spontaneous potential of 45 mV and short-circuit current of approximately 100 ua_{cm}-2. The active transport of sodium from mucosa to serosa surface is the main source of the observed potential. Chloride movement across this tissue is by passive diffusion combined with exchange diffusion. The movement of I^{-131} and Br^{-82} also behave in similar manner to Cl.

Direction of Current Research:

To study the movement of bicarbonate and CO^2 across the large intestine. Study the effect of inhibitors on sodium transport and on the chloride exchange diffusion. Project begun July 1956. Will end July 1958.

Part B Included: No

Form No. CRP-2
Oct. 1957

Serial No. MHI-146
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Cation Transfer Across the Red Cell Membrane

Principal Investigator: Edward T. Dunham

Man Years:
Total: 1
Professional: 1

Patient Days: None

Project Description:

Previously, a correlation in the decay of ATP and (active) K influx in the starved human red cell was reported. This encouraged an extension of this problem which included a comparison of active K influx with ATP utilization in the substrate-deprived red cell. After depletion of glucose (and hexose-phosphates), the only significant known energy reserves in this cell are 2,3 diphosphoglycerate (DPG) and ribotides. Pyruvate is no longer reduced. DPG disappearance and pyruvate appearance have been the principal measurements used in the estimation of energy-rich phosphate ($\sim$P) production. $\sim$P is also generated from ribose-phosphate, a product of ribotide degradation. $\sim$P production from this source has been estimated from the rate of total ribotide loss. This component, though small, is not insignificant, especially when the DPG reserve nears exhaustion. $\sim$P utilization has been determined by summing $\sim$P production and net $\sim$P loss. Active K influx was taken as total K influx minus "passive" K influx which remained after exhaustion of virtually all energy reserves. (This "passive" flux was insensitive to inhibitors). For a period of eight or more hours (37° C), beginning after hexose depletion and ending with the virtual disappearance of ATP, active K influx was proportional to $\sim$P utilization. However, since a reasonably good correlation also obtained between active K influx and $\sim$P production, additional information was needed. Conditions were introduced which it was hoped would distinguish between $\sim$P production and utilization and, at the same time, lend direct support to the presumed linkage of active cation transport to $\sim$P utilization. Mono-iodoacetate (IAA), which blocks the oxidation of triose-phosphate, was added to a portion of a hexose depleted cell suspension, followed by glucose addition to a sub-portion. Following a period of incubation, K influx was measured. Whereas only minimal inhibition resulted from addition of IAA alone, IAA (10^{-2} M) + glucose (100-200 mg%) produced a severe depression of K influx. Profound depletion of ATP resulted from hexose phosphorylation. Production of pyruvate from DPG was unaltered by IAA or IAA + glucose. This inhibition of active cation transport by glucose was interpreted as a competitive inhibition by hexokinase (and phosphofructokinase) utilization of ATP. This was supported by an observed rise in hexose-diphosphate of the appropriate magnitude after addition of IAA + glucose.

Direction of Current Research:

One series of experiments during the past year was undertaken to determine the influence of altered cell Na concentration on K influx in the hexose depleted red cell. Even when preloaded with 50 mEq Na (vs 8 mEq Na in control), only a minimal increase ($\leq$ 50%) in active K influx was observed in contrast to a marked increase ($\geq$ 200 %) under the same conditions in the presence of glucose. Net Na flux measurements pointed to a similar insensitivity of active Na efflux to increased cell Na after hexose depletion. Since it is known that glucose consumption and lactate production by human red cells are not substantially altered by alterations in cell Na and K, glucose consuming cells exhibit an apparent increase in transport efficiency with increased cell Na, whereas hexose depleted cells do not. $\sim$P utilization can be estimated from glucose consumption and lactate production. Apparent transport efficiency values derived from such estimates are in good agreement with those determined for hexose depleted cells in the presence of elevated cell Na ($\triangleq$ 40 mEq). However, at the physiological cell Na level of 8 mEq, the apparent efficiency is much lower for the glucose consuming cells. This suggests that glucose consuming cells may use only a part of the energy available for transport, drawing upon the remainder (which is ordinarily wasted) on demand. The above considerations, as well as others, suggest that such waste may take place via a phosphatase at the hexose-phosphate level. If increased transport utilization of $\sim$P results from increased cell Na, the resulting increase in the $\sim$P acceptor, ADP, might cause a displacement of the intermediate pools until a new steady state is reached in which a greater quantity of $\sim$P is utilized for transport and a correspondingly smaller quantity wasted by phosphatase action on the shrunken intermediate pool in question (e.g. hexose-diphosphate). If such a system is operative, a demonstrable alteration in that of the intermediate would be predicted under appropriate conditions. Current research is aimed at a resolution of this and associated problems.

Incidental Findings of Significance:

An attempt was made to determine whether steroids which enhance renal Na reabsorption would produce any significant reversal of transport inhibition by strophanthidin. Results with desoxycorticosterone and methyl-F-"F" were negative but aldosterone, the naturally occurring renal Na hormone, was less available in the quantity needed and has not yet been tried. Recently, however, a reversal of glycoside inhibition of human erythrocyte cation transport by aldosterone has been reported in the literature. The negative results for DOC and for methyl-F-"F" suggest that the aldosterone like activity of these synthetic steroids (in respect to the kidney) may derive from products of these compounds, rather than the compounds themselves.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Publication

Dunham, E. T. Parallel Decay of ATP and Active Cation Fluxes in Starved Human Erythrocytes. Federation Proc., 16: 1957.

Dunham, E. T. Linkage of Active Cation Transport to ATP Utilization. The Physiologist, 1957. (In Press)

Form No. CRP-2
Oct. 1957

Serial No. NHI-147
1 Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Method and Apparatus for Automatic Titration of Chloride

Principal Investigator: Ernest Cotlove

Cooperating Unit: Laboratory of Technical Development, Dr. Robert L. Bowman and Mr. Hillary Trantham.

Man Years:
Total: 3
Professional: 2
Other: 1

Patient Days: None

Project Description:

The effect of protein on the titration was shown to be due primarily to the presence of free sulfhydryl groups, and the magnitude of this effect was defined. For clinical use, the error in titration of plasma without precipitating protein was found to be negligible. With slight modification of the method, the instrument has been used to titrate sulfhydryl groups alone. Other factors influencing the accuracy and precision of the method have been defined further, and simple procedures developed for routine use. The advantages of the method and apparatus over standard analytic methods for chloride have been proven in routine use in a clinical laboratory. Information on construction of the instrument has been released to two interested manufacturers in order to make it generally available to clinical and research laboratories.

Direction of Current Research:

This project has been completed. The method is being applied to a study of tissue chloride.

Part B Included: Yes.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Publication

Cotlove, E., H. V. Trantham and R. L. Bowman. An Instrument and Method for Automatic, Rapid, Accurate and Sensitive Titration of Chloride in Biological Samples. J. of Laboratory and Clinical Medicine. To be published April, 1958.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Anion Transport Across the Gastric Mucosa

Principal Investigator: Dr. C. Adrian M. Hogben

Man Years:
Total: 8 months
Professional: 8 months

Patient Days: None

Project Description:

Both bromide and iodide are actively transported across the isolated gastric epithelium like chloride. Even when these halides are present in less than millimolar concentration, incorporation in or binding to protein does not account for their selective accumulation at the mucosal face.

The relative transfer of chloride across the two faces of the gastric mucosa has been studied in greater detail after reversible poisoning with dinitrophenol. The results of the experiments which were executed satisfactorially have not yet been analyzed.

The known dependence of gas secretion by the swimbladder upon carbonic anhydrase led to study of the isolated swimbladder because it is not immediately obvious that carbonic anhydrase would serve the function postulated in the parietal cell, i.e. the role of facillilating mobilization of disposable buffer. Many attempts to mount the swimbladder of the eel in a variety of media were unsuccessful. While the initial resistance would frequently be 2,000 ohms/cm^{-2}, the resistance would rapidly fall after any manipulation including exposure to 5% CO_2. No spontaneous potential or pH change was observed. Isolated stomachs from the same eels had a characteristic spontaneous potential and H^+ ion secretion.

While the gastric potential and acid secretion are intimately linked in several species we can not yet conclude that this is a necessary association. The isolated gastric mucosae of 2 teleost fishes, the eel and catfish, are similar to those of amphibia and mammals. In spite of the remarkable ease with which the stomach can be isolated from the elosmobranch dogfish no measurable potential or spontaneous H^+ secretion was obtained. Histamine did not induce

secretion. A single specimen of the cyclostome, Myxine, had neither a significant potential difference nor pH change across the isolated undifferentiated gut.

Part B Included: No.

1. Kidney & Electrolyte Unit
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Secretion of Substances into Bile

Principal Investigator: Dr. C. Adrian M. Hogben

Other Investigators: Dr. Lewis S. Schanker
Dr. Hermann Kurz

Cooperating Unit: Laboratory of Chemical Pharmacology

Man Years:
Total: 3
Professional: 3

Patient Days: None

Project Description:

This project was initiated in April 1957. Prompted by earlier reports in the literature that inulin appears in canine bile, the transfer of inulin and sucrose between plasma and bile was re-examined. The renal pedicles of the anesthetized rat were ligated and the bile duct cannulated. Beginning 1 hour after the intravenous injection of the C^{14}-labelled polysaccharide, three consecutive 90 minute samples of bile were collected. The radioactivity of these samples was compared to that of the terminal plasma sample. Plasma radioactivity was essentially stable during the collection of bile. The mean bile: plasma concentration ratios were 0.10 and 0.24 for inulin and sucrose respectively. These values suggest a significant porosity at some locus in the hepatobiliary system. Preliminary results with a third, smaller molecule, mannitol, indicate a bile-plasma concentration ratio of about 1.10.

Direction of Current Research:

a. Determine the inulin, sucrose and mannitol "space" of the liver.

b. Study the biliary secretion of other substances, especially weak organic electrolytes.

Part B Included: No.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Absorption of Drugs from the Human Stomach

Principal Investigator: C. Adrian M. Hogben

Other Investigators: Dr. Lewis S. Schanker
Mr. Dominick J. Tocco

Cooperating Unit: Laboratory of Chemical Pharmacology

Man Years

Total: 1
Professional: 8 months
Other: 4 months

Patient Days: None

Project Description:

Previous work from this laboratory described the absorption of drugs by the rat stomach and the secretion of parenterally administered drugs by the dog stomach. The results were consistent with the hypothesis that the gastric mucosa has the properties of a lipoid barrier, allowing rapid transfer of the unionized moieties of organic electrolytes while impeding the transfer of the ionized moieties. The present project, begun in January and completed in April, 1957, was undertaken to extend the study of gastric absorption to the human.

Solutions of various drugs were administered via Levine tubes to fasted normal subjects in the left supine position. Incorporation of phenol red in the solutions permitted estimation of the initial and final volumes and the correction for the concentration change due to dilution. The decrease in concentration of drug was determined at 10 minute intervals for 40 minutes. Acidic drugs, such as aspirin and secobarbital, were rapidly absorbed. Moderately strong bases, such as quinine and ephedrine, were poorly absorbed, while the very weak base, antipyrine, was fairly rapidly absorbed. These results, which parallel the previous study of absorption by the rat stomach, demonstrate that the ionization constant and the acidic or basic nature of a drug are important in determining gastric absorption. The human stomach is potentially an important site of absorption for many orally administered drugs if they are sufficiently soluble. Many drugs are absorbed from the human stomach more rapidly than ethyl alcohol, which heretofore was considered to be the unusual example of a drug which is absorbed by the stomach. Gastric absorption of drugs is both qualitatively and quantitatively similar in the human and rat. Project completed.

Part B Included: Yes

Serial No. NHI-150

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Publications:

Schanker, L. S., Shore, P.A., Brodie, B. B. and Hogben, C.A.M. Absorption of Drugs from the Stomach. I. The Rat. J. Pharmacol. and Exptl. Therap. (in press), 1957.

Hogben, C. A. M., Schanker, L.S., Tocco, D. J., and Brodie, B. B. Absorption of Drugs from the Stomach. II. The Human. J. Pharmacol and Exptl. Therap. (in press), 1957.

Form No. ORD-2
Oct. 1957

Serial No. NHI-751
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A:

Project Title: Absorptiam of Drugs by the Intestine

Principal Investigator: Dr. C. Adrian M Hogben

Other Investigators: Dr. Lewis S. Schanker
Mr. Dominick J. Tocco

Cooperating Unit: Laboratory of Chemical Pharmacology

Man Years:
Total: 2
Professional: 1½ months
Other: 8

Patient Days: None

Project Description:

This work continues the study of the relationship between the intestinal absorption of drugs and their physico-chemical properties. In a previous phase of this study, when solutions of various drugs were passed through the entire rat small intestine in situ a single time absorption rates of 0 to 60 per cent were observed. This technique revealed that absorption is a function of a drug's pKa, but it did not clearly distinguish relatively slow from very slow rates of absorption. Therefore the technique has been modified to magnify the differences between the rates of absorption of slowly and very slowly absorbed drugs.

The small intestine of the anesthetized rat was washed with a 1.0 ml drug solution containing a volume change indicator, phenol red or radioinulin. Then 30 ml. of fresh solution were continuously recirculated through the intestine for 3 hours. Total recoveries of phenol red and radioinulin were 98 ± 2 per cent. Drug absorption was calculated by correcting the concentration change of the volume change indicator. This technique clearly separated the absorption rates of drugs which are relatively slowly absorbed from those of drugs which are very slowly absorbed. The results support the previous finding that drugs are absorbed if they exist in solution in their unionized lipid-soluble form and that the intestinal mucosa is almost impermeable to the ionized form. Drugs like ephedrine and mecamylamine, which are highly ionized in the intestinal contents, were absorbed much more rapidly than quaternary ammonium bases and sulfonic acids which are completely ionized.

Direction of Current Research:

a. Ascertain whether lack of calcium ion in the intestinal lumen significantly modifies absorption.

b. Investigate the possible role of chelation in the absorption of certain drugs.

c. Investigate the effect of Diamox on absorption.

Part B Included: Yes

Serial No. NHI-151

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Publication

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 Exptl. Therap. (In press) 1957

Form No. ORP-2
Oct. 1957

Serial No. MCL-191
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Physiology of Congestive Heart Failure

Principal Investigator: James O.Davis

Other Investigators: Wilmot C. Ball,Jr. (1/2 yr)
Nicholas A. Yankopoulos(1/2 yr)

Man Years
Total 4
Professional: 2
Other: 2

Patient Days: None

Project Description:

Area I. To determine the mechanism of increased aldosterone secretion during heart failure.

Area II. To define the biochemical defect in the myocardium during heart failure.

Area I. Mechanism of Aldosterone Secretion during Heart Failure

Project I.

1. a. Title: Alterations in Aldosterone Excretion in Urine from Hypophysectomized Dogs with Thoracic Inferior Vena Cava Constriction and Ascites.

1. b. Investigators: J.O.Davis, R. C. Bahn, M.J.Goodkind and Wilmot C. Ball,Jr.

All experimental work on this project was reported last year. The paper was written and sent to press during past six months. This project began in spring, 1955 and was completed in spring, 1957.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Publication

Davis, J. O., Bahn, R. C., Goodkind, M. J. and Ball, W.C.,Jr. Alterations in Aldosterone Excretion in Urine from Hypophysectomized Dogs with Thoracic Inferior Vena Cava Constriction. Amer. J. Physiol. 191:329-338, 1957.

Davis, J. O. Some Aspects of the Physiology of Aldosterone. J. National Medical Association, 49:42-50, 1957

Goodkind, M. J., J. O. Davis, W. C. Ball, Jr. and R. C. Bahn. Alterations in Cardiovascular and Renal Hemodynamic Function Following Hypophysectomy in the Dog. Amer. J. Physiol. 188:529-534, 1957.

Ball, W. C. Jr., J. O. Davis and M. J. Goodkind. Ascites Formation Without Sodium Intake in Dogs with Thoracic Inferior Vena Cava Constriction and in Dogs with Right-Sided Congestive Heart Failure. Amer. J. Physiol. 188:577-582, 1957.

Davis, J.O., M.M. Pechet, W. C. Ball, Jr. and M. J. Goodkind. Increased Aldosterone Secretion in Dogs with Right-Sided Congestive Heart Failure and in Dogs with Thoracic Inferior Vena Cava Constriction. J. Clin. Invest. 36:689-694, 1957.

Goodkind, M.J.,W.C.Ball,Jr. and J.O.Davis. Effect of Chronic Hemorrhage on Urinary Aldosterone-Like Activity and Sodium Excretion in Dogs. Amer J. Physiol. 189:181-184, 1957.

Form No. ORP-2
Oct. 1957

Serial No. NHI-153
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A:

Project Title: Experimental Mitral Stenosis and Ascites Formation

Principal Investigator: James O. Davis

Other Investigator: David S. Howell

Cooperating Unit: Georgia Laboratories: Drs.W. F. Hamilton, Ellison, Hague, and Brown.

Man Years:
Total: 6 months
Professional: Bethesda Group: 3 months each

Patient Days: None

Project Description:

The experimental work on this project was done in 1953-54,. The study is a cooperative one between Dr. W. F. Hamilton and associates in Georgia and Drs.Howell and Davis. Both groups arrived at essentially the same conclusions in work carried out independently so it was decided to write a paper jointly. The findings are summarized in the following paragraph.

Extreme care must be taken in the production of experimental mitral stenosis in dogs by the method of controlled progressive constriction of the mitral ring in order to prevent the inadvertent constriction of the thoracic inferior vena cava and resultant elevation of the inferior caval pressure. After a report from the Georgia Laboratories that ascites formation was a common occurrence in dogs with experimental mitral stenosis produced by this technique, an attempt was made by the Bethesda workers to repeat these observations. From experiments performed in Bethesda, it was found that a stricture of the thoracic inferior vena cava was produced when the Georgia technique for production of mitral stenosis was used; this constriction provided an explanation for the ascites formation. These results were communicated to the Georgia workers who explored the two surviving dogs of their first series and found evidence which suggested that the inferior vena cava was patent even though the dogs were producing ascites profusely. Later, the Georgia group produced mitral stenosis in two additional dogs by the original technique and these animals gave unequivocal evidence that the stricture was present. Great care was then taken by the Georgia workers in placing the constricting ligature to avoid stricture of the orifice of the inferior vena cava. Of the thirteen dogs so treated only one

produced ascites and she had a small obstruction in the inferior vena cava. These last experiments by the Georgia workers as well as those of the Bethesda group suggest that ascites does not occur secondary to experimental mitral stenosis in dogs.

Funds, equipment and facilities were provided by the Georgia group for their experiments.

Part B Included: Yes

Serial No. NHI-153

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Publication

Hamilton, W. F., J. O. Davis, D.S.Howell, R.G.Ellison, E.E.Hague, and W.J. Brown,Jr. Experimental Mitral Stenosis and Ascites Formation. Amer. J. of Physiology 190:500-502, 1957.

Form No. OAP-2
Oct. 1957

Serial No. [redacted]
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Failure of Severe Adrenal Venous Congestion to Stimulate Increased Aldosterone Production

Principal Investigator : W. C. Ball,Jr.

Other Investigator: James O. Davis

Man Years:
Total:1 yr.
Professional: 8 months
Other: 4 months

Patient Days: None

Project Description:

This project was undertaken to determine whether a high venous pressure and the associated adrenal venous congestion would stimulate increased aldosterone production. The inferior vena cava was constricted immediately above the entrance of the adreno-lumbar veins into the inferior vena cava. Venous pressure below the constricting ligature increased 20 cm. of water. Aldosterone excretion remained unaltered and Na balance was maintained. It is concluded that adrenal venous congestion does not stimulate the adrenal cortex to secrete increased amounts of aldosterone. The work was begun and completed in spring of 1957.

Part B Included: Yes

Serial No. NHI-154

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Publication

Ball, W.C.,Jr. and J.O.Davis. Failure of Severe Adrenal Venous Congestion to Stimulate Increased Aldosterone Production. Amer. Journal of Physiology 191:339-341, 1957

Form No. CRP-2
Oct. 1957

Serial No. NHI-155
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Functional Changes During High Output Failure Produced by Hemorrhage in Dogs with Pulmonic Stenosis

Principal Investigator: James O.Davis

Other Investigators: M. J. Goodkind
W. C. Ball,Jr.

Man Years:
Total: 1
Professional: 1/2 yr.
Other: 1/2 yr

Patient Days: None

(This project began in fall of 1954 and was completed in spring of 1957) Dr. Goodkind relocated in July, 1956.

All data were reported last year but the paper was written and submitted during the last six months.

Part B Included: Yes

Part B: Publications

Davis, J. O., M.J.Goodkind, and W.C. Ball,Jr. Functional Changes During High Output Failure Produced by Daily Hemorrhage in Dogs with Pulmonic Stenosis. Circulation Research 5: 388-394, 1957.

Form No. CRP-2
Oct. 1957

Serial No. [illegible]-156

1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A:

Project Title: The Chronic Effects of Hypothalamic Lesions on Aldosterone and Sodium Excretion in Dogs with Experimental Ascites

Principal Investigator: James O. Davis.

Other Investigators: R. C Bahn,
W. C. Ball, Jr.

Cooperating Unit: Dr. R C. Bahn, Department of Pathological Anatomy, Mayo Clinic, Rochester, Minn.

Man Hours:
Total:4
Professional: 3
Other: 1

Patient Days: None

Project Description:

Observations have been made on 7 dogs with chronic lesions; these animals lived several weeks and were sacrificied. Subacute studies have been carried out on 3 additional dogs which lived 24-48 hours. Lesions were placed in the anterior, middle and posterior hypothalamus. In no instance did aldosterone output decline and Na excretion increase. Serial sections of the hypothalamus from each dog have been made and studied by Dr. Robert C. Bahn of the Mayo Clinic in order to define the precise location of the lesion. This study began in the spring of 1956.

The chronic study is complete but further observations are being made on the effect of acute lesions in anesthetized dogs in an attempt to locate or to exclude a hyperthalamic center for the regulation of aldosterone secretion.

Dr. Bahn performed detailed pathological studies which were supported by equipment and personnel at Mayo Clinic.

Part B Included: No

Form No. CRP-2
Oct. 1957

Serial No. [redacted]
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A:

Project Title: The Effects of a Body Cast upon Aldosterone and Sodium Excretion in Dogs with Experimental Ascites

Principal Investigator: James O. Davis

Other Investigator: Wilmot C. Ball, Jr.

Man Years:
Total: 1 1/2
Professional: 1
Other: 1/2

Patient Days: None

Project Description:

The present study was undertaken in an attempt to inhibit or prevent the loss of fluid into the peritoneal cavity by application of a body cast and to determine the effects on aldosterone and sodium output. Observations were made in 8 mongrel dogs. After a control period, abdominal paracentesis was performed and a body cast was applied. After 6-9 days the cast was removed and observations were made during a recovery period. During the period of the cast, a progressive increase in intra-abdominal pressure (indicated by a rise in inferior vena caval pressure) occurred, urinary aldosterone excretion dropped and sodium excretion increased. In 3 animals, sodium balance became negative. The increased sodium excretion was not attributable to elevated GFR because GFR was unchanged or reduced. Instead, the data support the interpretation that aldosterone secretion was decreased sufficiently to effect an increase in sodium excretion and, in 3 instances, net sodium loss. These observations are consistent with the general hypothesis that the rate aldosterone secretion is related to some factor or factors associated with fluid and electrolyte loss in clinical states of edema.

This project was begun in fall, 1956 and completed in June, 1957.

PART B Included: Yes

Serial No. NHI-157

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Publication

Davis, James O. and W. C. Ball, Jr. The Effects of a Body Cast on Aldosterone and Sodium Excretion in Dogs with Experimental Ascites American Journal of Physiology (in press) 1957

Form No. ORP-2
Oct. 1957

Serial No. AM-1638
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A:

Project Title: Acute Studies on the Mechanism of Aldosterone Secretion in Dogs with Thoracic Inferior Vena Cava Constriction

Principal Investigator: James O. Davis

Other Investigators: Nicholas Yankopoulos, Bernie Kliman, Ralph Peterson

Cooperating Unit: Lab. Arthritis and Rheumatism Br. NIAMD (Dr. Peterson and Kliman)

Man Years:
Total: 1 yr. and 8 mo.
Professional: 1 yr. and 8 mo.

Patient Days: None

Project Description:

This project was begun in Aug. 1957. The experimental design consists of a one hour control period in normal dogs and observations for 4-5 hours after thoracic caval constriction. Caval constriction has been found to stimulate aldosterone production within 15-30 mins. Present plans are to establish the degree of consistency with which aldosterone secretion can be increased by the technique of thoracic caval constriction. Interventions will then be introduced in an attempt to prevent or turn off the increase in aldosterone output and to define the factors immediately responsible for adrenocortical stimulation.

This project is being carried out in collaboration with Drs. Ralph Peterson and Bernie Kliman who developed the radioisotope method for measuring aldosterone and who are analyzing samples of adrenal vein blood for aldosterone and other steroid hormones.

Plans are to continue with this project as long as profitable possible mechanisms can be evaluated or until the factors leading to aldosterone secretion are clearly defined.

Funds and equipment in Dr. Peterson's laboratory are used in the chemical analysis of the steroid hormones. This equipment includes a scintillation counter, strip counter and scaler, full spectrum UV lamp, chromatographic tanks and equipment, radioactive tracers (C_{14} and H_3), crystalline aldosterone and other chemicals, glassware, etc.

Part B Included: No.

Form No. ORP-2
Oct. 1957

Serial No. AM-159
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A:

Project Title: Effects of acute hypothalamic lesions on aldosterone secretion in dogs with thoracic caval constriction and ascites

Principal Investigator: James O. Davis

Other Investigators: Nicholas Yanakopoulos
Bernie Kliman
Ralph Peterson
Bob Bahn

Cooperating Units: Br. Arthritis and Rheumatism, NIAMD (Drs. Peterson and Kliman)(Dr. Bahn, Department of Pathological Anatomy, Mayo Clinic, Rochester, Minn.)

Man Years:
Total: 2
Professional: 2

Patient Days: NOne

Project Description:

This work was started in August, 1957. The plan of the experiment is to study aldosterone secretion in anesthetized dogs with a thoracic caval ligature and ascites. After control measurements over a 1 hour period, a hypothalamic lesion is placed and the subsequent effects on aldosterone secretion measured. At the end of the experiment, the hypothalamus is removed and sent to Dr. Robert C. Bahn at Mayo Clinic where it is serially sectioned.

Studies have been made in only 3 dogs and data are available in only 2 of the 3 animals. In both dogs, placement of the hypothalamic lesion was followed by a marked drop in aldosterone and corticosterone output. However, marked circulatory changes occurred in both animals and one of the dogs had to be maintained on an infusion of L-norepinephrine to complete the experiment. Additional experiments are planned in which an attempt will be made to maintain the circulation better and to keep corticosterone output normal. Not even preliminary conclusions can be drawn from this small amount of information.

Plans are to explore thoroughly the possibility of a hypothalamic center for the regulation of aldosterone output.

Part B Included: No.

Form No. CRP-2
Oct. 1957

Serial No. NHI-160
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A:

Project Title: Isometric Tension Developed by Glycerol-Extracted Muscle from the Normal and Failing Myocardium

Principal Investigator: Nicholas Yankopoulos

Other Investigator: James O.Davis

Man Years:
Total: 2
Professional: 2

Patient Days: None

Project Description:

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 these latter are controlled. Glycerol-extracted myocardial muscle bundles were prepared by the method of Szent-Györgi (Biol. Bull. 96:140, 1949). The isometric tension is measured following addition of ATP by a Statham force transducer. All the technical details for this project have been worked out and data obtained on fiber bundles from hearts of one normal dog and two dogs with cardiac failure. No difference between the normal and the experimental material is apparent from this small amount of data. Project was begun in spring, 1957.

It is planned to study sufficient material to obtain a conclusive answer to this problem. Also, the effect of a digitalis glycoside on normal and failing muscle will be studied.

Part B. Included: No.

Form No. ORP-2
Oct. 1957

Serial No. HT-161
1. Kidney & Electrolyte Metabolism
2.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A:

Project Title: Chemical Characterization of the Contractile Protein in the Failing Myocardium

Principal Investigator: James O. Davis

Other Investigator: Nicholas Yankopoulos

Man Years:
Total: 2
Professional: 2

Patient Days: None

Project Description:

Studies are being conducted on the following parameters, in normal heart muscle and muscle from the failing heart: dry weight in percent wet weight FF dry weight, total protein in homogenate, total protein in soluble extract, actomyosin yield, and Na, K and Cl concentrations in muscle. The extracted actomyosin is characterized by measurements of ATP-ase activity and viscosity and by ultracentrifugal sedimentation analysis. Actomyosin is treated with ATP to obtain myosin and the sedimentation constants on both actomyosin and myosin studied at protein concentrations ranging from 1.8 to 8 mg. per cc. Plots of S_{20w} against protein concentration provide curves characteristic of the material subjected to centrifugation.

Observations have been made on muscle from 6 normal dogs and 3 dogs with heart failure. It has not been possible to obtain definitive data on all parameters from all the material because much of the time has been spent in working out the technical details. Also, all of the ultracentrifuge data has not been analyzed yet so that it is not possible at this time to arrive at any conclusions. There are, however, apparently no obvious gross differences between normal and failing muscle proteins. It remains to determine whether more detailed analysis of the data will reveal significant differences.

It is planned to complete the analysis described above and to obtain a large batch of more highly purified myosin and purified actin for further studies.

Also, the possibility of analysis of muscle structure by electron microscopy in the normal and failing heart is under consideration. With this technique it may be possible to study cardial muscle from patients with heart failure.

For this project we have used the ultracentrifuge and associated equipment in the Laboratory of Dr. Mihalyi.
Part B Included: No.

Form No. ORP-2
Oct. 1957

PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
Laboratory of Cardiovascular Physiology

Estimated Obligations for FY 1958

Total:	$207,060
Direct:	$139,029
Reimbursements:	68,031

Form No. CH-2
Oct. 1957

Serial No. NHI-162(c)

1. Laboratory of Cardiovascular Physiology
(Laboratory)
2. None
(Section)
3. Bethesda, Maryland
(Location)

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title:

Vasodilator Activity Present in Urine

Principal Investigators:

S. J. Sarnoff, K. E. Sussman, L. C. Sarnoff,
Jack Pierce, Evan Horning

Other Investigators:

Technical assistance of William Fisher

Cooperating Units: Dr. Jack Pierce and Dr. Evan Horning both
of the Laboratory of Chemistry of Natural Products

Man Years (calendar year 1957):		Patient Days (calendar year 1957):
Total:	6	39
Professional:	4	
Other	2	

Project Description:

During the past year, an attempt has been made to isolate the vasodilator activity present in urine and determine its physiological significance.

It was found that there are at least two substances in normal urine (both dog and human) which can dilate blood vessels; an organic base which is dialyzable and appears to be histamine, the second material which is non-dialyzable and a protein has biochemical properties which seem to indicate a similarity of this substance to Kallikrein which was described by E. Werle in 1930.

A more efficient method for the isolation of the non-dialyzable material has been developed yielding 70 - 80% recovery.

A comparison of patients with orthostatic hypotension and normal controls reveals a decreased urinary excretion of the non-dialyzable component in the patient group. This was interpreted as suggesting either a) that the vasodilator substance in human urine is in some way associated with autonomic function since its presence is greatly diminished in patients with an established autonomic defect and/or b) that the vasodilator substance in urine mirrors the relative average blood level of such a substance which, in turn, is an expression of one facet of the physiological response to blood pressure regulation.

More experiments will be conducted to determine what role the non-dialyzable dilator plays in regulating blood pressure. This will include patient studies, acute and chronic experiments in animals in which blood pressure will be varied.

An attempt will be made to determine the concentration of this material in the blood and to develop a spectrophotometric analysis for this determination.

Part B included Yes ☐ No ☒

Form No. OM-2
Oct. 1957

Serial No. NHI-163

1. Laboratory of Cardiovascular Physiology
(Laboratory)
2. None
(Section)
3. Bethesda, Maryland
(Location)

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title:

The development of a Bio-assay Technique for the Testing of Vaso-activity.

Principal Investigators:

Karl E. Sussman and L. C. Sarnoff

Other Investigators:

None

Man Years (calendar year 1957): Patient Days (calendar year 1957):

Total:	2	None
Professional:	2	
Other:	0	

Project Description:

The lack of reproducibility of the flow response to intra-arterially injected vaso-active agents had previously compromised its usefulness for this purpose. From the contour of some of the flow curves obtained it was suspected that lack of adequate mixing rather than changes in biological reactivity might account for the poor reproducibility. With this in view, the "intra-arterial" injections were made into a small multiple injection mixing cuvette (rotating magnet) placed in the femoral arterial flow line. Dilue dye studies demonstrated the improved mixing thus obtained and the bio-assay technique subsequently yielded gratifying reproducibility and adequate dose-response curve data.

This technique will continue to be used in the analysis of the vasodilator properties of urine.

Part B included: Yes ☐ No ☒

Serial No. NHI-164

1. Laboratory of Cardiovascular Physiology
(Laboratory)
2. None
(Section)
3. Bethesda, Maryland
(Location)

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title:

A Study of Factors Modifying the Duration and Mean Rate of Ventricular Ejection

Principal Investigators:

E. Braunwald, S. J. Sarnoff, W. N. Stainsby

Other Investigators:

None

Cooperating Units:

None

Man Years (calendar year 1957): Patient Days (calendar year 1957): None

Total: 3
Professional: 3
Other: 0

Project Description:

The object of these experiments was to systematically investigate the effect of a variety of factors influencing the duration and mean rate of ventricular ejection in the isolated, supported, non-failing canine heart preparation.

It was found that when stroke volume alone was increased, while holding all other parameters constant, the duration of ventricular ejection and mean rate of ejection are increased. An increase in heart rate at constant stroke volume decreases the duration of ejection per beat but increases the duration of ejection

Project Description (continued).

per minute as well as the mean rate of ejection. An increase in mean aortic pressure alone has little influence on either the duration or mean rate of ventricular ejection until markedly elevated mean aortic pressures are reached. Under such circumstances, duration of ejection is lengthened and the mean rate of ejection decreased. Hypothermia alone prolongs the duration of ejection, while sympathomimetic amines have the opposite effect. Unlike the non-failing heart, the failing heart exhibits an increase in the duration of ejection as stroke volume declines on the descending limb of its ventricular function curve.

Part B included Yes ☒ No ☐

Serial No. NHI-164

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications.

Publications:

1. Braunwald, E., Sarnoff, S. J., and Stainsby, W. N. The Determinants of the Duration and Mean Rate of Ventricular Ejection. Circ. Research. In press.

Honors and Awards: None

Serial No. NHI-166

1. Laboratory of Cardiovascular Physiology
(Laboratory)
2. None
(Section)
3. Bethesda, Maryland
(Location)

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title:

Quantitative Hemodynamic Analysis of
Valvular Insufficiency

Principal Investigators:

E. Braunwald, G. H. Welch, Jr. and S. J. Sarnoff

Other Investigators:

None

Cooperating Units:

None

Man Years (calendar year 1957): Patient Days (calendar year 1957): None

Total:
Professional: 3
Other: 0

Project Description:

The object of these investigations was to study the hemodynamic alterations resulting from the production of acute, metered, mitral and/or aortic regurgitation.

Two preparations have been devised in the dog with a complete circulation in which mitral or aortic regurgitant flow could be metered and the volume of regurgitation precisely controlled. The mitral insufficiency is created by permitting blood to flow out of the left ventricular apex through a prosthesis, through a Potter electroturbinometer and thence into the cannulated left atrium. The aortic insufficiency is created by permitting blood to flow from the thoracic aorta, through an electroturbinometer and into a prosthesis sutured into the apex

Project Description (continued).

of the left ventricle. In both preparations effective systemic flow is also metered. Pressures are measured simultaneously in the left atrium, left ventricle and aorta, and heart rate is recorded with a Waters cardiotachometer. Sixteen mitral insufficiency and twelve aortic insufficiency experiments have been carried out.

The results indicate that in:

I - Mitral Insufficiency:

a. The data obtained indicates that a large mitral regurgitant volume of the order of magnitude of four liters per minute could be sustained with only a minimal fall in forward cardiac output, and aortic pressure and associated with only a slight rise in mean left atrial pressure.

b. The left ventricular function curve is only slightly depressed in the presence of large volumes of mitral regurgitation (2 to 3 times effective forward flow).

c. The rise in left atrial pressure following an increase in systemic flow is substantially greater than the rise in left atrial pressure following a similar increase in mitral regurgitant flow.

II - Aortic Insufficiency:

a. The data from the aortic insufficiency experiments indicate that volumes of regurgitation are tolerated with considerable elevation of left ventricular end-diastolic filling pressure, but with relatively little rise in left atrial pressure.

b. The ventricular function curve is markedly lower after the production of aortic insufficiency than in the normal state; but if the regurgitant stroke work is added to the forward stroke work, the VF curve is even higher after aortic insufficiency is produced.

c. The differences between left ventricular end-diastolic and left atrial pressures could be diminished or almost abolished by inducing concomitant mitral insufficiency. However, the fall in left ventricular end-diastolic pressure was accompanied by a further diminution in effective stroke volume beyond that which had been produced by the aortic regurgitation alone.

Part B included Yes ☑ No ☐

Serial No. NHI-165

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications.

Publications:

1. Braunwald, Eugene, Welch, George H., Jr., and Sarnoff, Stanley J. Hemodynamic Effects of Quantitatively Varied Experimental Mitral Regurgitation. Circ. Research 5: 539-545, 1957.

2. Welch, George H., Jr., Braunwald, Eugene, and Sarnoff, Stanley J. Hemodynamic Effects of Quantitatively Varied Experimental Aortic Regurgitation. Circ. Research 5: 546-551, 1957.

Honors and Awards: None

Form No. CRD-2
Oct. 1957

Serial No. NHI-166

1. Laboratory of Cardiovascular Physiology
(Laboratory)
2. None
(Section)
3. Bethesda, Maryland
(location)

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Cardiac Energetics

Principal Investigator: S. J. Sarnoff, E. Braunwald, G. H. Welch, Jr. W. N. Stainsby and R. Macruz

Other Investigators: William Anderson, James Cox, William Fisher, Frank Ferry, and Joseph Miles.

Cooperating Units: None

Man Years (calendar year 1957): Patient Days (calendar year 1957): None

Total:	10
Professional:	5
Other:	5

Project Description: Activity in this long-term project for the past twelve months has consisted of a continuation of the studies outlined in the previous report; a comprehensive analysis of all available data and preparation for publication (see below). The data obtained increase the liklihood of the correctness of our position as outlined in previous reports and also that

a. The oxygen requirement of the heart is not meaningfully related to the work performed by it.

b. The oxygen requirement consistently correlates well with the Tension-Time Index (mean systolic pressure x the duration of systole).

c. These data provide an objective basis for understanding the clinical observation that patients with heart disease in which stroke volume and cardiac output are markedly elevated (mitral regurgitation, arteriovenous fistula, shunts) only rarely show overt evidence of myocardial

Project Description (continued).

hypoxia while patients with heart disease in which the myocardium is called upon to develop a large total tension (such as hypertension, aortic stenosis) do show such evidence in the form of angina pectoris.

An "amendment" to the basic Starling law concept (the energy of contraction is a function of end-diastolic fiber length) is being considered as a result of these data namely, that, while the end-diastolic fiber length determines the energy which can be put forth during the ensuing contraction, it is the nature of the contraction (tension or shortening) which determines how much energy is put forth.

The direction of current research is to acquire data with which to affirm or deny the above mentioned "amendment."

Part B included Yes [X] No [ ]

Serial No. NHI-166

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications.

Publications:

1. Sarnoff, S. J., Case, R. B., Welch, G. H., Braunwald, E. and Stainsby, W. N. Performance Characteristics and Oxygen Debt in a Non-Failing, Metabolically Supported, Isolated Heart Preparation. Amer. J. Physiol., January 1958.

2. Sarnoff, S. J., Braunwald, E., Welch, G. H., Case, R. B., Stainsby, W. N. and Macruz, R. Hemodynamic Determinants of the Oxygen Consumption of the Heart with Special Reference to the Tension-Time Index. Amer. J. Physiol., January 1958.

3. Sarnoff, S. J. and Braunwald. The Hemodynamic Determinants of Myocardial Oxygen Consumption. Encyclopedia Cardiology. In press.

4. Sarnoff, S. J., Braunwald, E., Welch, G. H., Stainsby, W. N., Case, R. B. and Macruz, R. Hemodynamic Determinants of the Oxygen Consumption of the Heart with Special Reference to the Tension-Time Index. The First Wisconsin Conference on Work and the Heart, May, 1957.

5. Braunwald, E., Sarnoff, S. J., Case, R. B., Stainsby and Welch, G. H. Hemodynamic Determinants of Coronary Flow: Effect of Changes in Aortic Pressure and Cardiac Output on the Relationship Between Myocardial Oxygen Consumption and Coronary Flow. Amer. J. Physiol., January 1958.

6. Welch, G. H., Jr., Braunwald, E., Case, R. B. and Sarnoff, S. J. The Effect of Nephentermine Sulfate (Wyamine) on Myocardial Oxygen Consumption, Myocardial Efficiency and Peripheral Vascular Resistance. Amer. J. Med. In press.

Honors and Awards: None

Serial No. NHI-167

1. Laboratory of Cardiovascular Physiology
2. None (Section)
3. Bethesda, Maryland (Location)

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title:

The Influence of the Site of Cardiac Stimulation on its Energetics

Principal Investigators:

Saul Winegrad, S. K. Brockman, J. R. Blinks, S. J. Sarnoff

Other Investigators:

None

Cooperating Units:

None

Man Years (calendar year 1957):		Patient Days (calendar year 1957)
Total:	4	None
Professional:	4	
Other	0	

Project Description:

It has been ascertained in preliminary experiments that -

a) Ventricular pacemaking results in a substantial depression of the ventricular function curve when compared with atrial pacemaking or conversely,

b) that at any given work level (compounded of specific flows, output pressures and rates) the ventricular filling pressure required is higher with ventricular than with atrial pacemaking and that under these circumstances

c) the oxygen utilization is higher with ventricular than with atrial pacemaking.

Part B included Yes ☐ No ☑

The direction of current research is to substantially amplify our data and develop, with the help of electrocardiographic information, an explanation of the observed phenomena.

Serial No. NHI-168(c)

1. Laboratory of Cardiovascular Physiology
2. None (Section)
3. Bethesda, Maryland (Location)

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title:

The Isolation and Characterization of a Cardiac-active Protein System in Serum.

Principal Investigators:

Stephen Hajdu, Edward Leonard

Other Investigators:

None

Cooperating Units:

Laboratory of Kidney and Electrolyte Metabolism

Man Years (calendar year 1957):		Patient Days (calendar year 1957):
Total:	2	150
Professional:	2	
Other:	0	

Project Description:

Initial investigations (thermolsbility, inactivation by organic solvents, retention by collodion membranes during ultrafiltration) suggested the protein nature of the system, and it was further revealed that more than one component was involved. One component (referred to as component L) binds strongly to the frog heart used for assay, and is the factor the concentration of which is elevated in hypertensive plasmas. The action of the system on the isolated frog heart is to restore the capacity to develop tension, to eliminate the staircase effect, and in the presence of a high concentration of component L to cause contracture. These actions can be reversed by washing the heart with several changes of physiological salt solution. The washing does not remove component L, but does remove a washable factor needed for activity of the system. Preliminary isolation procedures were designed to separate component L

Project Description (continued).

from the washable factor. The sensitivity of the proteins dictated the avoidance of organic solvents for separation; sodium sulfate is the most satisfactory precipitating agent.

Component L is now prepared from a serum containing the factor in high concentration, hypertensive human or normal dog serum being satisfactory. The protein is precipitated by dialysis of serum against 25% sodium sulfate, loss of the washable factor activity being assured by prior dilution of the serum. L activity survives in 50% glycerol at -20 degrees centigrade for many weeks.

Component L, when added to the heart, binds strongly and cannot be washed out over a period of many hours. A heart with component L bound is referred to as "sensitized". A sensitized heart with a large amount of component L bound serves as the assay system for the washable factor, addition of which then causes contracture.

During the attempted isolation of the washable activity, it was found that great losses occurred if room temperature dialysis was carried out against large volumes of calcium-free solutions. These losses can be prevented if calcium is present in all precipitating solutions. The washable fraction activity can be recovered practically without loss by precipitating the proteins of normal serum in 30% sodium sulfate containing 0.005M calcium chloride. This fraction contains all the serum globulins and a small fraction of the total albumin. (Albumin is not needed for the activity). Further purification of the washable activity yielded 2 protein fractions, neither of which alone has any effect on a sensitized heart, but which together reproduce the original activity. One fraction is precipitated from 0-17% sodium sulfate; the other, from 22-30%. The washable activity, then, is comprised of 2 protein fractions.

A number of experiments suggest that calcium plays an important role in the action of the washable fraction. Under the appropriate conditions, serum which is dialyzed against calcium-free solutions at 25 degrees centigrade loses washable activity. The loss can be prevented if dialysis is performed at 4 degrees, or if 5mM/L calcium chloride is present in the dialysis solution. Samples which have lost activity on dialysis can sometimes be reactivated by the addition of calcium. Free, ionic calcium does not of course substitute for washable activity. These results suggest that calcium may be bound to one of the proteins that comprise the washable fraction. Supporting evidence is found in the fact that this system can cause contracture of the sensitized heart in the absence of ionic calcium; and no other substance or system is known to induce heart muscle contraction in a calcium-free medium.

Part B included Yes [x] No [ ]

Serial No. NHI-168(c)

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications.

Publications:

1. Hajdu, Stephen, Leonard, Edward, and Akers, Robert P.: Action of Human Plasma on the Isolated Frog Heart. Observations on Subjects with and without Essential Hypertension. Circ. Research 5: 319-322, 1957.

Honors and Awards:

None

Form No. [illegible]

Serial No. NHI-169

1. Laboratory of Cardiovascular Physiology
(Laboratory)
2. None
(Section)
3. Bethesda, Maryland
(Location)

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title:

A New Isolated Atrium Preparation for the Study of Drugs Affecting Myocardial Contractility

Principal Investigators:

John R. Blinks

Other Investigators:

Charles Whitted (part time)

Cooperating Units:

None

Man Years (calendar year 1957):		Patient Days (calendar year 1957):
Total:	1½	
Professional	1	None
Other:	½	

Project Description:

The project, begun in November 1956, was concerned with development of experimental techniques and apparatus until August 1957. Since then, a series of experiments has been run to explore the potentialities of the method developed.

The preparation consists of the left atrium of the cat or rabbit, made into a closed sac by tieing the mitral ring around a cannula, ligating the pulmonary veins, and dissecting away all adjacent tissue. The atrium is filled with oxygen under pressure, and is mounted in a chamber filled with Krebs solution. It is stimulated at a constant frequency. In rapid succession, records are then made of the diastolic volume, stroke volume, and the

Project Description (continued).

pressure generated by "isometric" contractions, at a number of distending pressures. This permits the construction of pressure-volume curves, and "Starling curves" for both "isometric" and "isotonic" contraction from apparent ones produced by changes in frequency or diastolic fiber length.

It was observed that isolated left atria frequently developed a rapid spontaneous rhythm, and the necessity to suppress this in order to maintain a constant frequency of contraction prompted several experiments. It was found that the tendency toward auto-rhythmicity was increased by distention and by low potassium concentrations, decreased by increased potassium, pentobarbital, and reserpine. Reserpine pre-treatment effectively prevents the production of auto-rhythmicity by the sympathomimetic amines without significantly affecting their inotropic effects.

The results of the above experiments were as follows:

a) The diastolic pressure volume characteristics of the preparation were not changed by any of the drugs tested so far except the cardiac glycosides in toxic concentrations.

b) The "Starling curves" were not changed in shape, but were shifted up or down by drugs having positive or negative inotropic actions, respectively. The curves regularly exhibit a descending limb, and it is of special interest, from the theoretical point of view, that the peak of the curve, e.g., the diastolic volume associated with the strongest contractions, appears to be very nearly constant regardless of whether the curve is elevated or depressed.

A series of experiments is planned in which a large number of agents having inotropic actions will be examined for their effects upon the pressure-volume relationships, the "isometric" and "isotonic" Starling curves of isolated atria. Differences in the way in which the agents affect the various functions measured would indicate differences in their modes of action and might suggest the rational therapeutic combination of agents. It is also planned to evaluate the preparation for use in the assay of drugs for inotropic activity, comparing its performance with that of established methods.

Part B included: Yes ☐ No ☒

Form No. ORP-2
Oct. 1957

Serial No. NHI-170

1. Laboratory of Cardiovascular Physiology
(Laboratory)
2. None
(Section)
3. Bethesda, Maryland
(Location)

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title:

Heart Valve Replacement

Principal Investigators:

S. J. Sarnoff and S. K. Brockman

Other Investigators:

Frank Perry, Charles Whitted and Joseph Miles

Cooperating Units:

None

Man Years (calendar year 1957):		Patient Days (calendar year 1957):
Total:	5	None
Professional:	2	
Other:	3	

Project Description:

This project was reopened with a view to ascertaining whether the embolic phenomena which had precluded the clinical application of the apical-aortic anastomosis technique might be eliminated by the proper modification of the two points of attachment of the prosthesis at the ventricular apex and the aorta (see previous reports). It was found that impregnating a sleeve of stretchable dacron-orlon with elastic silicone and affixing this to the outflow end of the valve made it possible to perform an end-to-side anastomosis to thoracic aorta which appears to have overcome the above described difficulty. It has also been ascertained that the newly constructed silastic ball-valve produces substantially less hemolysis than the previously used ball-valve prosthesis.

Current efforts are aimed at making the ventricular (inflow) end of the prosthesis so that it conforms to the inner contour of the apex in order to obliterate the dead space in which the previously observed thrombosis was noted to arise and to have the entire surface exposed to blood covered with silastic.

Part B included (No)

Form No. CM
Oct. 1957

Serial No. NHI-171

1. Laboratory of Cardiovascular Physiology
(Laboratory)
2. None
(Section)
3. Bethesda, Maryland
(Location)

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title:

Effects of Blood Flow on Transcapillary Diffusion in Mammalian Skeletal Muscle

Principal Investigators:

E. M. Renkin

Other Investigators:

Frank Perry, William Anderson (part-time), and Alan Marks (June 1957 - August 1957). (The last-named was a participant in the summer student program. Donald McKnew, another summer student and James Brown also helped in much of the work described.

Cooperating Units: None

Man Years (calendar year 1957):
Total: 3½
Professional: 1
Other: 2½

Patient Days (calendar year 1957):
None

Project Description:

1. The principal phase of the study of blood flow and vascular tone on transcapillary K^{42} exchange in the isolated gracilis muscle of the dog was completed. In general, the conclusion was reached that the exchange rate is determined principally by two factors:

(a) the total blood supply.

(b) the extent to which the capillaries are open to the flow of blood.

Project Description (continued):

Experimentally, it was observed that sympathetic vasoconstriction led to a greater fall in K^{42} influx than could be attributed to the fall in flow alone, and that active (and reactive) hyperemia led to a greater increase in K^{42} influx than could be accounted for by flow alone. Thus a direct effect on the number of open capillaries is suggested, in agreement with histological study.

Attempts to study sympathetic vasodilatation were not successful.

This material will now be prepared for publication as one or more articles.

2. Work on the effects of sudden pressure changes on blood flow in skeletal muscles ended when Dr. W. N. Stainsby left on June 30, 1957, for the Physiology Department of the University of Florida at Gainesville. He is presently continuing this study.

3. Messrs. Marks, McKnew and Brown spent a large part of their time this summer working on the development of a new experimental preparation for me. This consists of the hind legs of a large bullfrog (R. catesbiana) perfused with oxygenated Dextran-Krebs solution in such a manner as to permit, ultimately, simultaneous measurement of K^{42} exchange and O_2 transport. The latter is to be measured by the Fick Principle, using a Clark O_2 electrode to measure venous O_2 tension (calculating content from the known solubility). Since the time available was limited, this development was not completed, but a good start was made.

(a) The perfusion method appears satisfactory. Provision must be made for removal of lymph accumulated under the skin (see section d, below).

(b) The Clark O_2 electrode works reasonably well, even at the low rates of flow encountered (0.2 ml./min.). It was not tested in a flow cell with blood, however. O_2 tension was recorded on a Sanborn channel via their low level D. C. amplifier (Chopper). Highly disturbing electrical "noise" which appeared intermittently was laboriously traced at last to 2 of the 3 chopper amplifiers in the department.

(c) The Beckman Industrial PH meter was adapted for

Project Description (continued):

recording venous PH on the Sanborn recorder, via a chopper amplifier. Problems relating to grounding and the design of a suitable flow cell still remain to be solved.

(d) Mr. McKnew made the interesting observation that what appears to be lymph may be collected by inserting polyethylene tubes under the skin of the perfused frog legs. Even without muscle movement or massage, it appears to be produced at an appreciable rate. This method may prove useful in studying lymph formation under very simple conditions.

Future plans are to be directed toward:

(a) the origin of lymph, and

(b) the time spent by blood in the capillaries during which diffusion occurs.

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PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
Laboratory of Technical Development

Estimated Obligations for FY 1958

Total:	$200,098
Direct:	$144,450
Reimbursements:	55,648

Proj. No. ORP-2
Oct. 1957

Serial No. NHI-172
1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Development of Freezing Point Depression Methods

Principal Investigator: Robert L. Bowman

Other Investigators: Lucy Samler (Summer employee)
David Shear (" ")

Cooperating Units: None
Dr. Jack Davidson-General Medicine
Dr. Norman Levinsky-LKEM

Man Years (Calendar Year 1957):
Total: .45
Professional: .15
Other: .30

Patient Days (Calendar Year 1957):
None

Progress During Past Year:

This has been a continuing problem since 1955, micro aspects began about January 1956.

Investigation of Peltier effect refrigeration systems for application to freezing point depression measurements was completed with the testing of a high efficiency couple prepared by RCA Laboratories and loaned to the laboratory for testing. Tests of this apparatus were conducted to determine if a reproducible time-temperature cycle could be produced so that time readings could be converted to temperature readings to allow interpolate of time of thawing between unknown and standards.

Current requirements of 50 to 100 ADC and requirement of constant temperature cooling water were considered impractical when compared with the conveniences offered by the cell. It was decided that this approach had little to offer over other methods of cooling.

A water bath type micro freezing point depression apparatus was built along the lines suggested by Ramsey (Ref.) but modified to utilize the time-temperature conversion for interpolation. The sample holder microscope system was also modified to hold several sample and unknown tubes. Testing of the apparatus is now underway in the Laboratory of K & E Metabolism.

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

Proj. No. ORP-2
Oct. 1957

Serial No. NHI-173
1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: A Counter-Current Model for Solute Transport and Diffusion in the Mammalian Kidney

Principal Investigators: Murray Eden

Other Investigators: Thomas J. Kennedy, Jr. (LKEM)
Robert W. Berliner (LKEM)
Norman Levinskey (LKEM)
Laura Guiffrida
Agnes Preston (LKEM)
Clara Adams (LKEM)

Cooperating Units: None

Man Years (Calendar year 1957):
Total: .37
Professional: .22
Other: .15

Patient Days (Calendar Year 1957): None

Progress During Past Year:

Studies of kidney functions, in particular, medullary functions have revealed certain physiological details, e. g., an ionic concentration gradient increasing from the cortex to the pelvis, that have not been explicated by presently available models.

A model has been proposed which postulates counter-current diffusion between the arterial and venous capillary systems in the renal medulla. Several mathematical models have been considered, all of which predict a gradient of solute concentration. Only the simplest models are capable of analytical solution, these models also predict that the gradient will be dependent on the square of the blood flow through the medulla.

Techniques have been devised so as to measure intra-medullary pH in vivo in dogs. It is anticipated that such experiments may serve to test certain of the inferences of the model. This project was started in January 1957.

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Individual Project Report
Calendar Year 1957

Direction of Current Research:

The more complex formulations of the model are being prepared for machine computation. Numerical results may then be used to make more detailed inferences regarding the roles of various physical parameters, such as flow rate, diffusion constants, etc.

The physiological experiments are continuing. An electrode suitable for embedment into the renal medulla will be fabricated and tested.

Part B included No.

Proj. No. ORP-2
Oct. 1957

Serial No. NHI-174
1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Development of a Model for the Explication of the Appearance Differences in pCO_2 between Plasma and Urine

Principal Investigator : Murray Eden
Other Investigators:-----Thomas J. Kennedy, Jr. (LKEM)
Robert W. Berliner (LKEM)

Cooperating Unit: None

Man Years (Calendar year 1957):
Total: .22
Professional: .22
Other:

Patient Days (Calendar year 1957):
None

Progress During Past Year:

It has been observed by workers in the Laboratory of Kidney and Electrolyte Metabolism and elsewhere that under certain conditions viz. a high buffer load, that the partial pressure of CO_2 in the urine may be 100 or more mm Hg higher than that in the plasma.

A mathematical model based on the known kinetics of the bicarbonate - carbonic acid - carbon dioxide system was devised, and a solution found for the differential equation, numerical computations were performed on the digital computer at the National Bureau of Standards. These results agreed with experimental results of studies of an in vitro system designed to test the hypotheses. It was found that in the presence of moderate to high buffer concentrations, the release of carbon dioxide from the solution may be delayed from 3 to 30 minutes, the delay time being increased as the buffer concentration increased. This project was started in January 1957.

Direction of Current Research:

A more complicated expression, including the catalytic effect of anions, has been derived. The numerical calculations will again require the use of a digital computer.

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Individual Project Report
Calendar Year 1957

While the results obtained agree well with the *in vitro* system, and agree qualtitatively with the physiological data, it is felt that other factors depending on mechanism of kidney function may be equally important in explaining these physiological data. (See Project Report- A Counter-Current Model for Solute Transport and Diffusion in the Mammalian Kidney).

Part B included yes ☐ no ☒

Proj. No. ORP-2
Oct. 1957

Serial No. NHI-175
1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

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 1957): Patient Days = None
Total: 1.30
Professional: .20
Other: 1.10

Progress During Past Year:

The general purpose of this project is to obtain information on the nature of the solid liquid interface in protoplasm, particularly its area and hydration structure. Projected was started in 1955.

The vacuum sublimation rate of frozen albumin and gelatin solutions of various concentrations has been measured. The drying rate of gelatin solutions is much slower than that of albumin of the same concentration by weight. This is in agreement with theoretical predictions. In order to correlate drying data with the size and number of ice crystals formed during the freezing, the dried specimens of gelatin and albumin have been embedded in paraffin sectioned and examined by phase microscopy. An unexpected result in this study was the extreme difficulty with which 10% gelatin was embedded by vacuum infiltration with paraffin in contrast with the relative ease with which tissue and albumin are embedded. This is interpreted as some kind of wetting phenomenon and is being investigated further.

The general theory necessary for interpreting the drying data has been developed beyond its previous state.

Direction of Current Research:

The compilation of drying data on various proteins and biological tissues is being continued. It is hoped that it may provide a useful estimate of the average state of aggregation of colloids in complex systems. The technique still needs to be refined so that it can be applied to very small samples - of the order of one microgram. At present this seems feasible. Another problem is to investigate the extent to which proteins are denatured by drying. It seems very probable that as water molecules are removed from a protein that irreversible bondings will occur between some of the freed bonds - and that at least from a physiological point of view cytoplasmic structure will be irreversibly denatured. One approach to this problem which is planned is to investigate the effects of freezing and dehydration on the physiology of red blood cells, particularly the sphering phenomenon.

Part B included: Yes

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Stephenson, J. L., Smith, G. W. and Trantham, H. V. Automatic Recording of Weight and Temperature for Vacuum Sublimation Studies, Rev. Sc. Inst., May, 1957, 28: 381-382.

Proj. No. ORP-2
Oct. 1957

Serial No. NHI-176
1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Development of Prosthetic Valves for Experimental Use in the Aortic and Mitral Areas

Principal Investigators: S. R. McCabe
R. L. Bowman

Other Investigators: H. V. Trantham
Alexander McInnes

Cooperating Units: Mr. Clarence Nemir, Nemir Incorporated
Dr. W. J. Kolff, Cleveland Clinic, Cleveland, Ohio
Dr. Frank Gollan, Veterans Hospital, Nashville, Tenn.
Dr. Frank Spencer, Johns Hopkins Research Department, Baltimore, Maryland
Dr. Glenn Morrow, Surgery, National Heart Institute

Man years (Calendar year 1957):
Total: .65
Professional: .40
Other: .25

Patient Days (Calendar Year 1957):
None

Progress During Past Year:

Dies of different sizes have been made and others started. Two valves, one for the mitral position and one for the aortic position are ready for a trial series in animals.

Valves have been molded in Polyvinyl chloride, silastic, elastomer of Kel-F with and without fillers. All materials are mechanically satisfactory but the vinyl chlorides are not suitable for long term use. They have been tested for short term use in dogs. The elastomer for Kel-F is difficult to mold and seems to have a greater tendency to 'flow' than does the more satisfactory silastic. The elastomers of polyurethane, and teflon (Viton A.) are under consideration.

Under the direction of Dr. Kolff of the Research Division, Cleveland Clinic, aortic valves have been placed below the coronary vessels. A cusp of the valve was imperfect and insufficiency resulted. A similar experiment was done at Johns Hopkins and a similar result obtained. Since this time the die has been revised. Valves designed for the Mitral area

PHS-NIH
Individual Project Report
Calendar Year 1957

have been inserted into dogs at the Veterans Administration Hospital, Nashville, Tennessee, under the direction of Dr. Gollan. The dogs survived several days and information regarding the housing of the valve was gained from these experiments. Dr. Clarence Weldon of the Heart Institute placed a valve in the mitral position. After the fifth day catheterization was performed, and the pressures recorded, in the left atrium and ventricle, were consistent with the normal. The dog was sacrificed and the area examined. A small amount of fibrin had gathered along a tear in an imperfect cust. Otherwise the valve was free of foreign material.

Direction of Current Research:

A study will be made by placing the valve in a series of dogs to see what further problems remain in the 'housing' of the valve. The valves have been used for pump oxygenators (Dr. George Clowes, Jr., City Hospital, Cleveland, Ohio) and further study will be continued for their use in pumps.

Part B included yes[ ] no[X]

Proj. No. ORP-2
Oct. 1957

Serial No. NHI-177
1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: An Analog Computer for the Analysis of Overlapping Distribution Functions

Principal Investigators: Murray Eden
Frank Noble
Joseph Hayes

Cooperating Unit: None

Man Years (Calendar year 1957):
Total: 1.52
Professional: 1.52
Other:

Patient Days (Calendar year 1957):
None

Progress During Past Year:

To develop procedures for the analysis of experimental curves resulting from the statistical distribution of a variable in a given molecular population, e. g., molecular absorption spectra and spatial diffusion patterns such as electrophoretic sedimentation curves. This project was begin March 1957.

It has been shown mathematically that any finite sum of components, each component being representable by a stable probability density distribution, e. g., the Gaussian distribution

has a unique decomposition.

However, in order to obtain this decomposition by mathematical analysis, Fourier transform methods are required which are exceedingly laborious and time consuming. It was decided to utilize a direct approach and to construct an analog simulator. With this device an operator varies the parameters of each component in some random way until he obtains a match to the experimental trace.

The computer simulates a spectrum by generating five periodic voltage waveforms of appropriate shape, each being individually adjustable in height, width, and position. The five waveforms are then summed and connected to a cathode ray oscilloscope where the

composite pattern is viewed. The repitition frequency is 60 cycles per second, hence the oscilloscope sweep is synchronized to the line frequency and phase. Individual position variability is obtained by shifting the phase of the 60 cycle sine wave which drives each individual channel. Width variability is obtained by forming a square wave from the input sine wave, integrating the square wave to obtain a triangular wave, passing the triangular wave through a variable gain amplifier, limiting the maximum positive excursion of the peak of the triangle to a fixed positive value and clipping the triangle at a fixed negative value. As the amplifier gain is changed, the output of the clipper is always triangular between fixed limits, but the width is continuously varied. This variable width triangle connects to a biased diode function generator which produces the desired distribution function. Height variability is obtained by variation of the gain of a following amplifier. The instantaneous sum is obtained by connecting all five channels to a resistive adding network.

Preliminary experiments on spectra of dinitrophenol and on certain synthetic mixtures of distributions have indicated good fits can be obtained by inexperienced operators with relatively little difficulty. However, it was found that in the case of curves exhibiting considerable overlapping of distributions, several different matches may be obtained on a single experimental curve.

Direction of Current Research:

In view of the questions of uniqueness of fit, it was felt that the likelihood of finding a single best fit would be considerably enhanced by improving the accuracy of the device.

The accuracy of solutions obtainable with this computer must certainly improve with refinement of the function generator component. We will attempt to produce function generators for both Gaussian and Cauchy-Lorentz distributions which are accurate to within one percent of full scale for both ordinate and area. When such generators are produced, we will redesign the remaining parts of the computer to be consistent in accuracy and increase the number of channels.

Two types of experiment potentially analyzable by this instrument are absorption spectrophotometry, in which the distribution is presumed to be either Gaussian or Cauchy-Lorentz, and techniques in which the curves are produced by a schlieren camera and are definitely Gaussian.

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With regard to the first type of experiment, two studies are planned. One is the analysis of infra-red spectra of water-primary aliphatic alcohol systems in which the effects of temperature on hydrogen bonding appear as changes in the relative intensity of several overlapping absorption bands. The other is an attempt to correlate absorption spectrum and chemical structure in the mono - and di-nitrophenol series, and absorption spectra and composition of mixtures of known compounds.

Two studies are planned with regard to the second type of experiment. One is the analysis of curves from electrophoresis and diffusion experiments on known synthetic mixtures of proteins; the other is the precise analysis of electro-phoretic patterns of pathologic (e. g., agammaglobulinemic) human sera.

Part B included yes ☐ no ☒

Proj. No. ORP-2
Oct. 1957

Serial No. NHI-178
1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Mechanism of Action of Metalloprotein Dehydrogenases

Principal Investigator: Joseph E. Hayes, Jr.

Other Investigators: None

Cooperating Unit: None

Man Years (Calendar year 1957):
Total: .20
Professional: .20
Other:

Patient Days (Calendar year 1957): None

Progress During Past Year:

Among the most important classes of enzymes in living tissue is the group consisting of dehydrogenases which require pyridine nucleotides as electron acceptors. A number of these are known to be zinc metalloproteins, whose integrity as such is essential to their enzymatic activity. There is in addition evidence that in the case of the alcohol dehydrogenase of bakers' yeast there is one atom of metal for each of the four active sites of the enzyme molecule, so situated that their interaction with a metal-complexing agent interferes with binding of coenzyme to the apoenzyme. Also, there are grounds for suspicion that removal of the zinc leads to extensive degradation of the protein molecule.

It is planned to investigate these phenomena with especial reference to elucidation of the function of the zinc atom in the catalytic action.

Many metal-chelates are known to be fluorescent. It is hoped that binding of substrates or coenzymes to the zinc-enzyme can be studied by this means. Existing methods for direct demonstration and kinetic study of enzyme-coenzyme complexes depend on changes in absorption spectra. Such changes do not occur with yeast alcohol dehydrogenase, making an alternative method such as this necessary.

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1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
Calendar Year 1957

Studies by standard physical methods are planned to investigate degradation of the protein on removal of the zinc.

It is expected that correlation of such studies with studies of coenzyme binding by other methods (e. g. ultracentrifugal separation) and kinetics of the overall reaction will yield information concerning the function of the zinc and the component elementary reactions of the overall scheme.

This project began with the arrival of the investigator in this laboratory in February 1957.

Because of unavailability until recently of a spectrophotofluorometer, difficulties with intrinsic instability of the enzyme, and primary involvement of the investigator in another project, (A Computer for Determining Wave Number, Intensity and Line Width of Maxima in the Infra-Red Spectrum). Progress to date is negligible.

Direction of Current Research:

The current project is directed toward an understanding of the nature and mechanism of the catalytic action exerted by members of this class of enzymes, through measurement by locally developed instrumental methods of interaction of an enzyme with its coenzymes and substrates and studies of the kinetic behavior of the complexes thus formed.

Part B included yes[ ] no[x]

Proj. No. ORP-2
Oct. 1957

Serial No. NHI-179
A. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Biochemical Effects of Ultrasonic Waves

Principal Investigator: Alfred Weissler

Other Investigators: None

Cooperating Unit: None

Man Years (Calendar year 1957):
Total: .5
Professional: .5
Other:

Patient Days (Calendar year 1957):
None

Progress During Past Year:

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. Among the known effects: lysis of erythrocytes, the killing and disruption of various cells and viruses in suspension, the neurosurgical production of brain lesions, and a differential rate of destruction of oxidative and phosphorylative activity of enzymes. Generally, these effects are found only if the ultrasonic intensity is great enough to produce cavitation. One aim of these studies is thus a better understanding of the way in which cavitation causes oxidation and depolymerization; for example, are oxidants (such as H_2O_2) formed by the recombination of hydroxyl or other free radicals produced by a presumed electric discharge in the cavities, or do they result from thermal activation of dissolved oxygen by local temperatures of several hundred degrees attained during the adiabatic compression of the collapsing bubbles, or is some other mechanism involved.

Preliminary experiments indicate that dissolved oxygen is necessary for the formation of H_2O_2 in ultrasonically-irradiated distilled water; little, if any, H_2O_2 is produced in water which contains helium as the only dissolved gas. Because of this, and also the finding that moderate concentration of hydroxyl radical scavengers (such as acrylamide and formic acid) do not reduce the peroxide yield, it is unlikely that H_2O_2 is produced principally by the reaction $2\,OH = H_2O_2$.

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Individual Project Report
Calendar Year 1957

Another observation is that small amounts of ozone are produced by ultrasound in distilled water containing dissolved oxygen.

Direction of Current Research:

The possibility of detecting hydroxyl and perhydroxyl (HO_2) radicals directly by electron paramagnetic resonance methods will be explored. Also, oxygenated and deoxygenated solutions of enzymes such as catalase and dehydrogenase will be irradiated, and the loss of enzymatic activity will be correlated with spectrophotometric and molecular weight changes. The extent of the analogies between sonochemistry and x-radiation chemistry will be investigated, as one aspect of the broad problem of the conversion of sound energy into heat, light, and chemical energy.

Part B included yes ☐ no ☒

Proj. No. ORL-2
Oct. 1957

Serial No. NHI-180
1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Development of a Probabilistic Model for Growth

Principal Investigator: Murray Eden

Other Investigators: Laura Guiffrida

Cooperating Unit: Dr. Hale Trotter, Princeton University, Princeton, New Jersey.

Man Years (Calendar year 1957):
Total: .27
Professional: .12
Other .15

Patient Days (Calendar year 1957):
None

Project Description:

To construct a minimal information, stochastic, two-dimensional model of a growing organism and to study the properties of the model. Since the model is in a certain sense an analogy to biological growth, the results on the mathematical model may give some insight into the minimal information content required for biological growth. Project began prior to Principal Investigator's arrival on duty in June, 1955.

Progress During Past Year:

Samples of 256 cells and 512 cells, respectively, have been prepared by the Monte Carlo method described in earlier reports by the Institute for Advanced Study computer facility.

A paper was prepared dealing with the machine computation aspects of the study and delivered at a Symposium on Computer Techniques in Biology and Medicine, sponsored by the Institute of Radio Engineers in New York City on March 4.

Direction of Current Research:

A thallophyte, Prasiola sp. has been found which bears a gametophylic thallus having a striking resemblance to the results of the theoretical model. Attempts will be made to obtain this organism in order to ascertain whether the morphogenesis of this structure resembles the process postulated by the model.

Part B included Yes [X] No [ ]

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Bonner, J. T. and Eden, M., The Form of the Frequency Distribution Curve of Cell and Nuclear Sizes. Experimental Cell Research, II, 265 (1956).

Honors and Awards relating to this project:

None

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Oct. 1957

Serial No. NHI-181
1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Development of a Micro Glass Electrode

Principal Investigator: Murray Eden

Other Investigators: Laura Guiffrida

Cooperating Units: None

Man Years (Calendar year 1957):
Total: .97
Professional: .22
Other: .75

Patient Days(Calendar Year 1957):
None

Project Description:

To develop a glass electrode small enough to be embedded into living organisms for some length of time. To ascertain how small a glass electrode can be made that will function as an adequate sensing element for the measurement of pH. Project began in June, 1955.

Progress During Past Year:

Various methods of insulating all except the lowest 50 microns of the micro glass electrode have been attempted, these have included coating with varnishes, and vacuum evaporating aluminum, silver, gold and silicon monoxide. None of the methods have been found to be satisfactory.

Somewhat larger electrodes have been fabricated by sealing a small amount of the conducting glass to a lead glass capillary and then pulling the bubble out to a fine point 50 - 100 microns in diameter. These electrodes are being used in a study of the pH response of the renal medulla *in vivo*. (See report of T. J. Kennedy, Jr., LKEM).

Direction of Current Research:

The method described in the previous paragraph is being extended to the fabrication of smaller size electrodes.

A further modification consisting of a method of pulling the microcapillaries of conducting glass within an envelope of lead glass so that the two glasses fuse together a short distance from the tip will be investigated.

Part B included yes [ ] no [x]

Proj. No. ORP-2
Oct. 1957

Serial No. NHI-182
1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Development of a Sound Velocity Detector for Gas Chromatography

Principal Investigators: Frank W. Noble
John L. Stephenson

Other Investigators: Eugene McKelvey (summer employee)
Marion Sharpless
Hillary Trantham

Cooperating Units: None

Man Years (Calendar Year 1957):
Total: 1.00
Professional: .35
Other: .65

Patient Days (Calendar Year 1957):
None

Progress During Past Year:

The need for a more sensitive and stable detector is discussed in the report "Development of Radio frequency Ionization Detector for Gas Chromatography" by Dr. Robert L. Bowman. The development of a sound velocity was started in July of this year. For constant pressure and ratio of specific heats, the velocity of sound is universely proportional to the square root of the density of the gas. A device which actually measures sound velocity can be calibrated to indicate gas density. One possible advantage of the use of sound is that the small pressures involved presumedly will not alter the physical or chemical properties of the gas mixture. We have built a model which utilizes a sound frequency of one megacycle and a path two centimeters long and one centimeter in diameter. A quartz crystal is mounted in each end of the chamber. One crystal is driven by a stable oscillator, while the other receives the sound from the first and feeds an amplifier. The relative phase of the transmitting and

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receiving crystal voltages is determined and is interpreted in terms of sound velocity or gas density. At the present time the sensitivity is of the order of $1:10^5$ mole fraction for a gas of MW 100 in helium.

Direction of Current Research:

The sensitivity of this instrument must be increased by at least one hundred times in order to represent a significant improvement over existing methods of detection. Two possibilities are being investigated. One is to obtain greater phase sensitivity in the measuring circuit of the present equipment. The other is the use of frequency multiplication of both the transmitted and received signals to increase the phase difference between them. The combination of both methods which yields the maximum sensitivity consistent with acceptable stability will be found.

Part B included. No

Proj. No. ORP-2
Oct. 1957

Serial No. NHI-183
1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Development of Methods for Measurement of Blood Flow

Principal Investigators: Robert L. Bowman
Frank W. Noble

Other Investigators: H. V. Trantham

Cooperating Units: None

Man Years (Calendar year 1957):
Total: .93
Professional: .33
Other: .60

Patient Days (Calendar Year 1957):
None

Progress During Past Year:

The aim of this continuing project is to produce an efficient method of measuring blood flow with a minimum distrubance of the system being measured. The method utilizing Nuclear Magnetic Resonance is still considered to have the best promise for fulfilling the requirements. The electronic equipment necessary to produce spin echo signals has been completed, but preliminary tests have shown that in spite of the fact that this system is capable of operation in inhomogenous magnetic fields that we still have not a sufficiently powerful magnet to make a successful spin echo test. In view of the necessity of a more elaborate magnet than would be practical for this type of system, the spin echo system has been temporarily suspended in favor of continuing work on the absorption method that has so far shown the greatest promise. Experiments with various types of circuits for picking up the nuclear magnetic absorption signal from the flowing stream are still being tested. The defect of this system at the moment is the requirement of the large magnet to produce the magnetic field. Systems are under consideration for producing this field in a pulse fashion with solenoids or devices which would be less cumbersome than the large magnet presently required.

Direction of Current Research:

The investigation of the possible application of the Nuclear Magnetic Resonance system to measurement of blood flow, hydration, and the analysis of the alkali metals utilizing the broad band simple system will be continued as time becomes available.

Part B included. No

Proj. No. ORP-2
Oct. 1957

Serial No. NHI-184
1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Development of a Self-Balancing Potentiometer

Principal Investigators: John L. Stephenson
Frank W. Noble

Other Investigators: Geraldine W. Smith
Marion Sharpless

Cooperating Units: None

Man Years (Calendar year 1957):
Total: .42
Professional: .17
Other: .25

Patient Days (Calendar Year 1957)
None

Progress During the Past Year:

The electronic amplifier used in this apparatus was modified slightly to incorporate a cathode following stage. A stabilized 700 volt power supply was also built for the photomultiplier used. It was found that the best signal to noise ratio was obtained when the tube was operated at the highest light level which would not cause excessive fatigue of the photocathode.

Direction of Current Research:

Except for publication the project is completed.

Part B included --- no

Proj. No. ORP.-2
Oct. 1957

Serial No. NHI-185
1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Mathematical Investigation of Biological Transport Problems

Principal Investigator: John L. Stephenson

Other Investigators: Marion Sharpless

Cooperating Units: None

Man Years (Calendar Year 1957):
Total: .40
Professional: .20
Other: .20

Patient Days (Calendar Year 1957)
None

Progress During the Past Year:

A number of biological problems have been investigated from a probabilistic point of view. These include interchange of a label between N compartment systems and interchange of a label between vascular and extra-vascular fluid compartments. It has been found that these problems can be usefully analyzed in terms of transport probabilities which define the probability that a particle initially at one phase will be found at another t units of time later. In particular a very general relation has been found which relates the transport through a capillary bed of a substance which exchanges with the extra-vascular fluid compartment to the transport of one which does not. Utilizing this theoretical result - if the outflow curves are experimentally known it should be possible to obtain the law governing exchange between the capillary bed and the extra-vascular fluid compartment.

Direction of Current Research:

It is planned to continue investigation along same lines.

Part B included - no

Form No. ORP-2
Oct. 1957

Serial No. NHI-186

1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Physics of Ultra-Rapid Freezing of Water, Colloidal Solutions and Protoplasm

Principal Investigator: John L. Stephenson

Other Investigators: Geraldine Smith
Artrice Valentine
Marion Sharpless

Cooperating units: None

Man Years (calendar year 1957)
Total: 1.70
Professional: .45
Other: 1.25

Patient days: None

Project Description: (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. Project was begun in 1954.

Progress During Past Year: Recording techniques have been further developed. A procedure for the manufacture of microthermocouples has been devised in which a glass capillary tube is pulled down around a 1 mil constantan wire, giving it a very thin coating of glass. The tip of the wire is then exposed, and a layer of copper is evaporated on to it. The completed thermocouple has a diameter of about 2 mils and a response time of about 1 millisecond. In addition to galvanometer amplifier used for following the thermocouple response has been somewhat improved. (See report on self balancing potentiometer).

The earlier work on effects of pressure freezing has been written up for publication.

A major activity during the past year has been to set up the facilities and to acquire the skills necessary for doing our own electron microscopy. Towards this end a Philips E. M. 75 microscope has been rented, installed, and its operation has been learned.

Equipment for doing our own preparation of biological specimens has also been set up, and the routine procedures of methacrylate embedding, then sectioning, preparation of grids, etc. have been learned. At present we are beginning to get reasonably satisfactory micrographs of specimens prepared by standard procedures.

Direction of Current Research: The primary program in the next year is to correlate data obtained from calorimetric studies with electron microscopic data as to occurrence size and number of ice crystals. This will both help to evaluate freezing and drying as a method of fixation for electron microscopy and will give fundamental information about the kinetics of ice crystal formation in colloidal systems during rapid freezing.

Part B. included Yes X

Part B. Honors, Awards and Publications

Publications:

1. Gersh, I., Isenberg, I., Stephenson, J. L., Bondareff, W., Submicroscopic Structure of Frozen-Dried Liver Specifically Stained for Electron Microscopy. PartI, Technical Anat. Record. May, 1957, 128: 91-111.

2. Gersh, I., Isenberg, I., Bondareff, W., Stephenson, J. L. Submicroscopic Structure of Frozen-Dried Liver Specifically Stained for Electron Microscopy. Part II, Biological. Anat. Record, June 1957, 128: 149-171.

Form No. ORP-2
Oct. 1957

Serial No. NHI-187

1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Construction of a Small Pump Capable of Working the Pulmonic and Peripheral Systems Simultaneously

Principal Investigator: S. R. McCabe

Other Investigator: William Tribble (Technical)

Cooperating units: Philip Joram and George P. Marsden - Medical Arts Section, Scientific Reports Branch
Dr. Stanley J. Sarnoff, Laboratory of Cardiovascular Physiology, National Heart Institute

Man Years: (calendar year 1957)
Total: .9
Professional: .65
Other: .25

Patient Days: None

Project Description:

Progress during past year: The pump described in previous report has been made. A modified pump of small proportions has been tested. It is roughly spherical and is two and a half inches in diameter. During animal tests it is connected to I. V. C. and S. V. C. by way of the azygos vein and the right atrium. The 'right' side of the pump ejects blood which is directed by a connector through the wall of the outflow tract of the right ventricle into the pulmonary artery. Blood is collected from the left atrium and pumped into the descending aorta. This is done through the 'left' side of the pump.

A new activating system has been designed and made. Its central feature is an electric clutch. Its magnetic field is controlled by an electrical pulse. The length of time of the 'on' signal controls the stroke output of the pump. The number of signals per minute control the pulse rate. The system is set apart from the pump and its force is transmitted through a tube containing fluid. The pump has the advantage that its controls can be placed at any desired distance and an accurate pressure curve can be generated with ease of control.

A test run with the pump showed that the animal's lungs could be used as the oxygenator. An A/V difference of 4% was obtained and no edema of the lungs was apparent after an hours run. The animal was kept at full flow for two and a half hours. All reflexes remained present. There was no visible sign of hemolysis after this period of

time. Femoral pulse pressures were almost identical to the pulses generated by the animals own heart.

Direction of Current Research: The pump is being redesigned to facilitate its attachments to the animal system. A yet smaller pump will be made. A yet smaller activating system will be made with the intention that the pump may be of advantage not only for by-pass experiments but also for long term use experiments with the stopped heart.

Part B. included: No X

Form No. ORP-2
Oct. 1957

Serial No. NHI-188

1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Development of Radio Frequency Ionization Detector for Gas Chromatography

Principal Investigator: Robert L. Bowman

Other Investigators: Arthur Karmen and David Shear (summer employee)

Cooperating units: Laboratory of Cellular Physiology & Metabolism, Metabolism Section (Arthur Karmen)

Man Years (calendar year 1957) Patient days: None
Total: .4
Professional: .25
Other: .15

Project Description:

Progress During Past Year: (Began July 1, 1957). An evaluation of reports in the literature of gas chromatography indicated that progress in extending the sensitivity and utility to biological problems (the determination of the fatty acids in vascular deposits) was limited by the availability of a more sensitive and stable detector. Several systems reported by others to show promise were tested for further development. Ionization gauge at low pressures operated at a voltage to discriminate against carrier gas and read only the "unknown". The behavior of this detector in our hands led us to believe that the basic assumption of discrimination was in question and several technical difficulties were introduced, such as constant proportioning "leaks" to provide sampling, contamination of the gauge filament, and elements causing excessive drift.

A summer student employee (Eugene McKelvey) working with Dr. John Stephenson studied the properties of an isolated gauge to test the basic assumption that helium ionization could be prevented while the esters could be ionized and read as signal. When his observations indicated, as we suspected, that the separation was inadequate this system was abandoned.

In the interim a DC corona discharge method was tested but had several disadvantages such as excessive destruction of the sample and deposition of products on the electrodes.

The most promising systems currently under consideration are a sonic method reported separately by Mr. Noble of this laboratory, and

a R. F. discharge system based on considerations of the probable mechanism of the origin of D. C. potentials in radio frequency excited gas discharges. These potentials are well known to be highly stable and form the basis of a gauging device utilized in a micro inch inspection gauge. The prior application utilizes the potentials in a different manner but the information that they could be maintained in highly stable form for many hours suggests that at least the basic phenomenon has great stability and leaves only the specific sensitivity to be developed. Several forms of this detector have been constructed and tested for sensitivity by providing known concentrations of fatty acid esters from a gas saturating and handling manifold constructed for the problem. Testing has also been carried out utilizing a chromatograph column run at room temperature. Current practice has shown 1: 10^5 mole ratio to be the practical limit of detectors in use. The R. F. detector has shown excellent response at concentrations at least an order of magnitude better with stability suggesting that it could be improved further. Testing methods have been hampered by the difficulty of preparing and confirming standards.

Direction of Current Research: Further development of the detector head and suitable circuits for control and recording will be developed.

Part B. included No X

Form No. ORP-2
Oct. 1957

Serial No. NHI-189

1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Development of an Ultramicroanalytic Method for Sodium and Potassium Determination in Micropuncture Samples.

Principal Investigator: Robert L. Bowman

Other Investigators: Mildred Williams Skarda (Jan.-May 1957)
Willard C. Whitehouse

Cooperating units: Thomas J. Kennedy (LKEM) Collaborating on testing the methods

Man Years (calendar year 1957)
Total: 1.2
Professional: .2
Other: 1.

Patient days: None

Project Description:

Progress During Past Year: The development of methods for determination of the metals in question is continuing at a reduced pace due to the absence of trained technical assistance. The outstanding advantage of this particular system is its very high sensitivity. The use of a special red sensitive system involving the image converter reported previously has allowed us to make measurements of cesium and rubidium, as well as the previous mentioned metals. Chloride bands have been observed but the sensitivity seems to be several orders of magnitude less than that for the sodium and potassium sensitivity. The reproducibility of rereading any particular sample had been very poor until the current method of rotating the tube was instituted. Rotation of the tube at 1800 rpm while it is being excited in the microwave cavity has largely eliminated the problem of special positioning of each individual tube. A method of exciting the entire tube in such a fashion as to allow the entire optical output to enter the optical

system was also tried but did not give satisfactory results. This method involved the excitation of the sample tube in a coaxial line rather than in a microwave cavity so as to permit the use of a larger viewing window. A satisfactory tuned line for excitation of the electrodeless discharge was successful but the ease with which the tubes could be lighted was considerably less than in the cavity. These tuned lines however, will be further considered as a means of excitation of the halide lines as they probably will require cooling which will not be compatible with the rotating tube system.

In order to compare the effectiveness of the flame photometer to perform this same determination, we set up an old style Beckman burner and Bausch and Lomb monochrometer with the newly available infra-red photomultiplier tube as the pick-up unit. This system allows the finest atomizer to be used to deliver very small quantities of samples over an extended period of time, provides a large aperture dispersing system and utilizes an effective photomultiplier measuring circuit. With this system samples of 8 hundredths of a cubic centimeter can be added over a period of 15-20 seconds time.

The sensitivity of this system has been such as to make it extremely difficult to dilute the sample adequately without becoming involved with concentrations of impurities in very best grades of distilled water which are equivalent to the expected concentrations in the diluted sample. Samples of demineralized distilled water prepared in this laboratory have compared favorably with a sample of high purity conductivity water obtained from the Bureau of Standards. The experience gained from comparing these two methods has led us to conclude that refinements of the flame photometry in the sense of improving flame stability and sample preparation, and the use of higher optical efficiency which is available in the use of interference filters instead of the less efficient dispersing system will allow the flame photometer to compete with the electrodeless discharge. This latter system has a great advantage in not requiring any dilution and permitting analysis of several materials at once without consuming the sample.

Both of these methods are essentially ready to be applied and require only the collaboration of Investigators utilizing micropuncture techniques to further evaluate their performance.

Direction of Current Research: The ultimate aim of this problem is to construct and test an instrument capable of analyzing these metals in the range of concentrations and sample size required for micropuncture techniques.

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Oct. 1957

Serial No. NHI-190

1. Laboratory of Technical Development
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Development of Fluorometric and Phosphorimetric Methods for Biological Application

Principal Investigator: Robert L. Bowman

Other Investigator: Willard C. Whitehouse

Cooperating units: None

Man Years (calendar year 1957)
Total: .70
Professional: .20
Other: .50

Patient days: None

Project Description:

Progress During Past Year: Project began 1951. With the completion of the ultraviolet SPF and its relegation to the position of a standard method of analysis, it became apparent that extension of its sensitivity into the red and infra-red end of the spectrum would extend the scope of the system to several classes of biologically important materials, notably the porphyrins red sensitive photomultiplier tubes have recently become available and are currently being tested for their applicability. One tube available from RCA type - has satisfactory characteristics. In order to extend the utility of the instrument to determine emission spectra of phosphorescent materials, higher light intensity is necessary and high intensity flash lamps have been acquired and a system for storing and integrating the emission is being developed. To make flash excitation of phosphorescence practical a system of integration and storage can be used to produce a simple rapid spectral analysis. The method uses a special photo-conductive paper (electrofax) developed for facsimile transmission. The paper is charged to a high potential by means of a corona discharge and after suitable exposure to the spectrum it is "read out" by means of a vibrating capacitor probe. This system has been constructed and tested. The sensitivity resolution and convenience appear to be satisfactory.

Direction of Current Research: Research for the extension of fluorescence and phosphorescence methods will continue as new materials become available. Development of the phosphorescence method for application to frozen glassy samples in non aqueous media with flash excitation will continue and electron beam excitation will be tested in the future.

Part B. included Yes X

Part B. Honors, Awards and Publications

Honors: The principal investigator has been elected to Chairman of an ASTM Committee to set up standard practice recommendation in fluorescence instrumentation and education.

Publication:

1. Duggan, D.E., Bowman, R. L., Brodie, B. B. and Udenfriend, S. A Spectrophotofluorometric Study of Compounds of Biological Interest, Archives of Biochemistry and Biophysics, Vol. 68, No. 1, May 1957, Academic Press, Inc.

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NATIONAL HEART INSTITUTE

Summary Budget Data
General Medicine and Experimental Therapeutics Branch

Estimated Obligations for FY 1958

Total: $1,105,640

Direct: $412,656

Reimbursements: 692,984

Form No. CRD-2
Oct. 1957

Serial No. NHI-191
1. GMET
2. Clin. Endo.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the Determination of Urinary Aldosterone.

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

Other Investigators: Harold Schedl, M.D., Ph.D., Moses Middleton

Cooperating Units: None

Man Years:
Total: .9
Professional: .3
Other: .6

Patient Days: None

Project Description: A variety of chemical reactions were tested in attempting to develop a more convenient method for chemical determination of urinary aldosterone. Specific reactions studied since December, 1956, have been aimed at determining the reactive carbonyl groups, especially the 3 keto. Two reactions showing promise were the 2, 4 dinitrophenylhydrazine method, and a new reaction involving the U.V. spectrophotometric determination of a salicyloyl hydrazone. Various separative schemes - ion exchange, adsorption columns, partition and paper chromatography - were tested. It was finally decided to employ the salicyloyl hydrazide reaction, for it showed (a) greater specificity (Δ^4-3 ketosteroids react to form compounds with characteristic U.V. spectra) and (b) better separation (the derivative could be separated quantitatively from the reagent).

Presently, we are in the final evaluation stages of this technique, working out the best chromatographic separation of aldosterone from urine and analyzing some urines for aldosterone in parallel with the dog bioassay technique.

Part B included Yes [ ] No [X]

Form No. CRP-2
Oct. 1957

Serial No. NHI-192
1. CMET
2. Clin. Endo.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Conversion of Progesterone-C^{14} to Aldosterone by the Perfused Adrenal Gland.

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

Other Investigators: Harold Schedl, M.D., Ph.D., Diana Redd

Cooperating Units: This work was done in collaboration with Dr. George Rosenfeld, USN, but no funds were supplied from that source.

Man Years:
Total: .6
Professional: .4
Other: .2

Patient Days: None

Project Description: 101 μc Progesterone-4-C^{14} (40 mg) were perfused through calf adrenal glands in the apparatus of Rosenfeld. An extract of the perfusion fluid was subjected to fractionation by paper chromatography and analysis by autoradiography and paper strip counting. Results indicate much less conversion to aldosterone-4-C^{14} than the originally estimated 0.5%. Infra-red microscope measurements on 10-15 γ quantities of aldosterone from paper chromatograms showed poor spectra due to paper blank material.

Our present experiments are designed to prove that our material is aldosterone by a "constant isotope ratio" method. Using tritium ring labelled aldosterone obtained by the enzymatic hydrolysis from aldosterone acetate, by Peterson, we shall chromatograph mixtures showing (we hope) constant specific activity.

Part B included Yes/ / No /X/

Form No. ORP-2
Oct. 1957

Serial No. NHI-193 (c)
1. GMET
2. Clin. Endo.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Water Loading in Normal and Cirrhotic Subjects.

Principal Investigator: Harold Schedl, M.D., Ph.D.

Other Investigators: Frederic C. Bartter, M.D., Catherine S. Delea, Diana Redd

Cooperating Units: None

Man Years:
Total: .6
Professional: .5
Other: .1

Patient Days: 120

Project Description: In nine studies in normal subjects, water in the amount of 2% of the body weight caused a 5-15 milliosmols per liter fall in serum osmolality. This was accompanied by a diuresis with a free water clearance of 5-12 ml/min.

In four studies in two cirrhotics, a comparable fall in serum osmolality was produced, but the accompanying diuresis showed a free water clearance of only 1-3 ml/min.

The relation of this difference to increased antidiuretic hormone action will be studied.

Part B included Yes / / No /X/

Form No. CRP-2
Oct. 1957

Serial No. NHI-194
1. GMET
2. Clin. Endo.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Determination of plasma testosterone.

Principal Investigator: Harold Schedl, M.D., Ph.D.

Other Investigators: Philip S. Chen, Jr., Ph.D., Diana Redd

Cooperating Units: None

Man Years:
Total: 1.3
Professional: .5
Other: .8

Patient Days: None

Project Description: Double labeling, using tritium and C^{14} acetates, is being investigated for the analysis of testosterone in plasma. The method appears to have the specificity and sensitivity needed for dog spermatic vein blood testosterone levels. In the normal dog, testosterone levels of 5-15 γ/100 ml. are found in spermatic vein blood.

The physiology of release of testosterone and effects of Follicle Stimulating Hormone will be studied. The method may prove to be of sufficient sensitivity for studies of testosterone metabolism in man.

Part B included Yes /___/ No /X/

Form No. CRP-2
Oct. 1957

Serial No. NHI-195
1. CMDT
2. Clin. Endo.
3. Bethesda, Md.

PHS-410
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Colorimetric Determination of Magnesium.

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

Other Investigators: None

Cooperating Units: None

Man Years:
Total: .2
Professional: .2
Other: None

Patient Days: None

Project Description: This is a continuation of some studies begun in 1951 at Rochester on a colorimetric method for serum and urine magnesium. A sensitive dye which changes color from amber to violet at pH 12 in the presence of magnesium was found. The major problem confronted at that time was the instability of the color complex. Propylene glycol has been found to stabilize the color and a method of obtaining increased sensitivity was found, but several new factors have developed.

The primary problems now are (1) H_2O inhibits the color, and (2) the complex does not follow Beers law.

Part B included Yes [ ] No [X]

Form No. CRP-2
Oct. 1957

Serial No. NHI-195
1. GMET
2. Clin. Endo.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on Parathyroid Hormone.

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

Other Investigators: Moses Middleton

Cooperating Units: None

Man Years:
Total: .5
Professional: .1
Other: .4

Patient Days: None

Project Description: The studies on parathyroid hormone (begun March 19, 1957) are not easily classified together, but are listed together since the general goals are (a) a sensitive assay technique, preferable one which could be used to measure parathyroid titers in human blood; (b) mechanisms of parathyroid action; and (c) chemical nature and constitution of the parathyroid hormone. We have not had time to devote undivided attention to this problem and it has been a part-time project. Assay was first tried by infusing serum from Bailey into a parathyroidectomized dog. The control zero phosphorus excretion was unchanged.

Another experimental approach has been to infuse calcium into a normal dog containing radioactive (P^{32}) red cells, attempting to show PO_4-ester release of P^{32}-inorganic PO_4 under the influence of a calcium infusion. Two experiments, unfortunately on the same dog, were equivocal. Although no increased specific activity of serum inorganic PO_4 was found, the dog showed very little increase in inorganic PO_4 after calcium gluconate, i.e., 1.5 mg. % PO_4 before and 1.7 mg. % PO_4 after administration of 2.8 gms. calcium gluconate (5 mg. Ca/min. for one hour.) It is planned to repeat this experiment.

Assay studies have been tried on laying hens, infusing Parathyroid Injection (Lilly) in saline into one leg vein and comparing PO_4 excretion from the two kidneys. Results thus far are disappointing in that there was a large biological variation in hens and only two birds out of 17 showed a clearcut difference between the two sides. However, parathyroid infusion always did produce a PO_4 diuresis in hens showing an initially stable PO_4 output.

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Individual Project Report
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It has been decided to (1) supplement the diet of these hens with oyster shell; (2) to use capons, roosters, or parathyroidectomized hens (rather difficult, since the glands lie on the vessels above the heart within the rib cage) to try to find variables which regulate chicken response to parathyroid hormone.

Part B included Yes / ___ / No / X /

Form No. CRP-2
Oct. 1957

Serial No. NHI-197
1. GMBT
2. Clin. Endo.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Study of the Circulation of Large Molecules Through Tissues.

Principal Investigator: Leroy E. Duncan, Jr., M.D.

Other Investigators: Katherin P. Buck

Cooperating Units: This work was done in collaboration with Mr. Jerome Cornfield, Division of Research Services, but no funds were supplied from that source.

Man Years:
Total: 2
Professional: 1
Other: 1

Patient Days: None

Project Description: The project was begun two years ago. It is a continuing project designed to provide an understanding of the processes involved in the movement of molecules the size of albumin and larger from the blood stream, through the tissues, and back into the blood stream directly or via the lymphatic system.

The work has so far been limited to studies on rabbits. The molecule to which the most study has been devoted is serum albumin labeled with radioiodine. Animals are injected with radioalbumin, killed at intervals, and determinations of the radioalbumin in various tissues made. From an appropriate mathematical analysis of these data, the rates of transfer of radioalbumin from serum to specific tissues and the rates of transfer out of the specific tissues can be computed. This general method is applicable to the study of transfer rates of molecules larger than albumin.

A study of the circulation of albumin through aorta, skin, tendon, sclera, cornea, and muscle has been completed and is ready for publication. Considerable differences exist in the transfer rates of albumin into and out of these specific tissues and in the equilibrium tissue-serum concentration ratios.

The transfer rates we have determined can be interpreted in terms of current concepts of filtration and diffusion of solutes into tissues at capillary and lymphatic exchange sites. This formulation suggests possible explanations of the differences in the transfer rates observed.

The transfer rates from the movement of albumin into tissues can be converted into the type of capillary permeability coefficient determined by Pappenheimer for smaller molecules. Previously, this type of permeability coefficient could be determined only for molecules considerably smaller than albumin.

From the serum data alone, by means if simplifying assumptions and appropriate mathematical analysis, it is possible to compute average transfer rates for rabbit tissue. These average transfer rates are, of course, the weighted averages of the transfer rates of the various tissues in the body. The average transfer rates fall in the range of the specific transfer rates we have obtained.

There are data available in the literature which, though they have not been used for that purpose, can be manipulated to yield rates of movement of radioalbumin into skin and muscle. The appropriate calculations yield transfer rates for human skin very similar to those for rabbit skin. The transfer rates for rabbit muscle are somewhat faster than for human muscle.

A second study which has been completed but not yet written up, is one of the effect of dicumarol on capillary permeability. There are several reports in the literature that dicumarol increases the permeability of tissues to albumin. We have studied this by the methods outlined above and have found no effect of dicumarol on the transfer rates of radioalbumin.

There are two main areas where this type of study might throw light on heart disease. The processes influencing the rate of movement of albumin into and out of tissue are of importance in the control of the volume of interstitial fluid and thus in the formation of edema. One of the current theories of the origin of atherosclerosis is that lipoproteins filter from plasma into arterial tissue where they are trapped. Studies of the transfer of macromolecules into various tissues are of importance to an understanding of the problems involved and to an evaluation of the filtration theory.

Currently, we are studying the transfer rates of radioalbumin into major body tissues so that the disappearance from the plasma of intravenously injected radioalbumin can be better understood in terms of its removal into specific tissues. The next contemplated study using these techniques is a study of the effect of expansion of the blood volume on the transfer rates of albumin into tissue and the effect of edema on the transfer rates of albumin out of tissue.

Another line of work will be the study of the use of larger molecules than albumin for obtaining information on capillary permeability and tissue trapping.

A third line of study proposed is an evaluation of methods for determining capillary permeability to large molecules in man.

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Part B: Honors, Awards, and Publications

Duncan, Leroy E., Jr., Cornfield, Jerome, and Buck, Katherin, The Circulation of Iodinated Albumin Through Aortic and Other Connective Tissues of the Rabbit, Circulation Research. In press.

Form No. CRC-2
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Serial No. [illegible]
1. GMET
2. Clin. Endo.
3. Bethesda, Md.

NIH-[illegible]
Individual Project Report
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Part A.

Project Title: The Effect of Estrogens on the Metabolism of Adrenal Corticosteroids.

Principal Investigator: Ivor H. Mills, M.D.

Other Investigators: Frederic C. Bartter, M.D., Catherine S. Delea

Cooperating Units: None

Man Years: .8 Patient Days: 100
Total:
Professional: .6
Other: .2

Project Description: The metabolism of hydrocortisone was shown by Martin and Mills to be altered during pregnancy in such a way that the plasma hydrocortisone is markedly elevated, whereas the urinary excretion of its metabolites is within the normal range. It was suggested that the increased production of estrogens during pregnancy might be responsible for the altered hydrocortisone metabolism.

Cortisone acetate, 100 mgs., was administered once daily intravenously to obtain a relatively stable plasma hydrocortisone level. Daily determinations of plasma hydrocortisone and urinary corticoid excretion were made for five to fourteen days. Estradiol benzoate, 2 mgs., was given daily intramuscularly for seven days in addition to the cortisone acetate, and the daily plasma hydrocortisone and urinary corticoid determinations were continued.

The results on three normal controls and one man with cirrhosis produced the same general pattern. During the control period the plasma hydrocortisone fell steadily in two subjects and remained constant in the other two. When estrogen was administered, there was a marked rise in plasma hydrocortisone in three individuals. In the cirrhotic subject, the rise was temporarily extremely high. One normal subject showed a transitory rise followed by a slow decline. The urinary excretion of corticoids did not give such a clear-cut pattern because the recovery in the urine of administered cortisone acetate, as Porter-Silber chromagens, is only 5-12% of the dose when it is given intramuscularly. However, the tendency was for the urinary excretion to change in the reverse direction to that of the plasma values. These data suggest that estrogen does alter the metabolism of administered cortisone acetate.

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The study is being extended to cover changes in adrenal production of hydrocortisone and its pool size, in individuals not being given exogenous steroid, when estrogen is administered.

Part 3 included Yes / / No / X /

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Serial No. NHI-199 (c)
1. GMET
2. Clin. Endo.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Action of Parathyroid Hormone.

Principal Investigator: Pacita Pronove, M.D.

Other Investigators: Frederic C. Bartter, M.D., Harold Schedl, M.D., Ph.D., Catherine S. Delea, Cecilia P. Kirby, Elaine K. Kohn

Cooperating Units: None

Man Years:
Total: 2.4
Professional: .9
Other: 1.5

Patient Days: 950

Project Description: The general purpose of this research is (a) to study the physiology of the parathyroid gland and the mechanism of action of the parathyroid hormone, and (b) to clarify the diagnosis of hyperparathyroidism. The four types of tests described in the progress report of December, 1956, were carried out in 21 subjects not suspected of parathyroid disease and in 19 subjects suspected of parathyroid disease. Progress to date may be summarized as follows:

1. Tm of phosphorus: In four normal subjects the Tm of phosphorus was more than 3 mg/min. In seven subjects with proven hyperparathyroidism, the Tm of phosphorus was more than 3 mg/min in three, and less than 3 mg/min in four.

2. Response of urine phosphorus to intravenous calcium. Calcium infusion produced a fall in urine phosphorus in nine normal subjects with "rebound" phosphaturia. It did not produce a fall (or rebound) in six patients with proven parathyroid adenoma. In the normals, the serum phosphate rose with a resultant marked fall in phosphate clearance. In the patients with parathyroid adenoma, the phosphate clearance did not change.

3. Response of serum and urine calcium and serum phosphorus to amphogel. In four subjects with parathyroid adenoma, amphogel produced the following results: (a) the urine calcium rose above 200 mg/day; (b) the serum phosphorus fell below 2.0 mg. %; and (c) the serum calcium rose above 11 mg. %. In four normal subjects, amphogel had none of these effects.

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4. In four subjects ammonium chloride (130 mEq/day for five days) did not lower the serum CO_2 content below 24 mEq/liter. This was taken to indicate that the patients did not have renal tubular acidosis as an alternative cause of renal stones.

The studies described above are being carried out in subjects suspected of hyperparathyroidism. The results are under analysis.

Part B included Yes /——/ No /X/

[illegible] Clin. Endo.
[illegible] Bethesda, Md.

NHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

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

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

Other Investigators: Edward G. Biglieri, M.D., Ivor H. Mills, M.D., Pacita Pronove, M.D., Catherine S. Delea, Cecilia P. Kirby, George W. Smith

Man Years: Patient Days: 1000
Total: 3.8
Professional: 2.3
Other: 1.5

Project Description: The general purpose of this research is to evaluate the role of the adrenal cortex in the sodium retention of edema:

A. To investigate the stimuli to aldosterone secretion

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

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

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

Studies on the stimuli to aldosterone secretion were continued. The blood volume was expanded with albumin in normal subjects, in subjects with cirrhosis, and in subjects with hypoproteinemia. Aldosterone excretion was consistently lowered by this procedure.

The blood volume was decreased in normal subjects with phlebotomies and the red cells later reinfused. Twelve normal subjects were so studied. In ten, phlebotomy produced an increase in aldosterone excretion. When sufficient time was allowed to elapse there was then a spontaneous fall in aldosterone excretion. When the red cells were reinfused, there was a further fall in aldosterone excretion in ten of the twelve subjects. Dogs were studied by measuring adrenal venous aldosterone during the induction of acute changes in circulating volume with similar results.

The role of aldosterone was investigated in patients with nephrosis, with cirrhosis with ascites, with postural hypotension (with Dr. Henry Wagner), and with primary aldosteronism. An attempt was made to induce a state resembling primary aldosteronism by giving 2-methyl-fluoro-hydrocortisone to normal subjects.

In nephrosis, it was found that aldosterone excretion was higher on a low sodium intake, but fell transiently when the sodium intake

was increased, but at the expense of considerable expansion of extracellular fluid volume, and that urinary aldosterone fell simultaneously with urinary protein when meticorten was given and diuresis promptly ensued.

Two patients with Laennec's cirrhosis and ascites were studied to determine the response to albumin given intravenously, potassium depletion, ACTH, and delta-1-fluoro-hydrocortisone. Intravenous albumin produced marked depression of aldosterone excretion with diuresis. Potassium depletion produced similar falls in aldosterone excretion without diuresis, as did delta-1-fluoro-hydrocortisone. Results with ACTH are still being collected. Patients with postural hypotension showed little rise in aldosterone excretion after sodium deprivation, despite moderate loss of weight. Three patients with primary aldosteronism* showed high urinary levels of aldosterone during control studies. These were shown not to be responsive to changes in sodium intake. In all three, it was found that serum potassium and bicarbonate became normal with low sodium intake and despite elevated aldosterone levels. The renal handling of potassium and hydrogen by these patients was compared with that in six normal subjects receiving short courses of 2-methyl-fluoro-hydrocortisone. The results of this study are being analyzed.

Renal clearances with measurement of potassium, hydrogen, and sodium excretion were carried out on two patients with Addison's disease before and during administration of aldosterone intravenously. Marked spontaneous decrease of sodium excretion made the effect of aldosterone difficult to interpret. The procedure resulted in increased excretion of potassium and hydrogen.

Several compounds which are suspected of the ability to suppress adrenal cortical secretion have been tested by infusion into anesthetized dogs during collection of adrenal venous blood. Several of these compounds can depress hydrocortisone secretion almost totally. Studies in progress will determine whether their effect on aldosterone secretion is comparable.

* One studied in conjunction with Walter Reed Army Medical Center.

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Part B. Honors, Awards, and Publications

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

Bartter, F.C., Biglieri, E.G., Pronove, P., and Delea, C.S. Aldosterone Secretion in Man, Acta Medica Scandinavica, 1957. In press.

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Serial No. [illegible]
1. GMBT
2. Clin. Endo.
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Individual Project Report
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Part A.

Project Title: Structure-function Relationships in Anabolic Steroids.

Principal Investigator: Harold Schedl, M.D., Ph.D.

Other Investigators: Frederic C. Bartter, M.D., Catherine S. Delea, Margaret Martin, Cecilia P. Kirby, and Gaynelle S. Greene.

Cooperating Units: None

Man Years:
Total: 1.5
Professional: .3
Other: 1.2

Patient Days: 700

Project Description: Three patients with osteoporosis and six normal subjects were given steroids while on balance regimen. 19-nor compounds were compared with 19-methyl compounds and with methyl testosterone. In three studies on osteoporotic subjects, 9α fluoro-11β-hydroxy-17α-methyl testosterone was given; in two, its action was compared with that of methyl testosterone. (Two of these studies were begun in 1956.)

The 19-nor compounds (17α ethyl-, 17α vinyl-, and 17α ethinyl-19-nor testosterone) appeared to be as potent as 17-methyl testosterone in inducing nitrogen retention in normal young women. The corresponding 19-methyl compounds (17α ethyl, 17α vinyl, and 17α ethinyl testosterone) appeared to be inactive in this respect.

The 19-nor compounds induced more sodium retention than the corresponding 19-methyl analogs. 9α-fluoro-11β-hydroxy-17α methyl testosterone appeared to be four to five times as potent as 17-methyl testosterone in inducing protein anabolism and bone formation.

The studies described above are under analysis. Further studies will depend on results.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Bartter, Frederic C., Osteoporosis, American Journal of Medicine, 22:797-806, May, 1957.

Oct. 1957

1. GMET
2. Exp. Therapeutics
3. Bethesda, Md.

PMS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Hypersensitivity Studies in Humans

Principal Investigator: Halla Brown (and staff of the George Washington University Allergy Clinic) and T. Phillip Waalkes, M.D.

Other Investigators: None

Cooperating Units: G. W. University

Man Years:
Total: .40
Professional: .25
Other: .15

Patient Days: None at the Clinical Center (1½ days per week at the G. W. Allergy Clinic)

Project Description: Animal experiments indicate that serotonin may be a factor in allergic reactions since it is released from platelets, and possibly tissues during anaphylaxis. Reserpine depletes all the body depots of serotonin and, in rabbits, lowers platelet histamine. Therefore, reserpine therapy to reduce the body stores of serotonin to a minimum might be beneficial in patients with sensitivity to ragweed pollen. Eighty patients with this specific allergy are being used in this study which is still in progress at the George Washington University Allergy Clinic.

This is the first study of hypersensitivity in humans; others are planned depending on the outcome of the present work. In addition, further investigation of the role of serotonin in human allergy is in progress. Also a thorough search into the role of histamine, by a study of its newly found metabolites in urine, is planned. Applications of these studies to hypersensitivity diseases affecting the cardiovascular system are being considered.

Part B included: Yes [ ] No [X]

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Serial No. NHI-204 (C)
1. GMET
2. Exp. Therapeutics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Metabolism of Amino Acids in Man

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

Other Investigators: John D. Davidson, M.D.; Louis Gillespie, M.D.; H. Weissbach, Ph. D.; Dr. Chozo Mitoma and Dr. S. Udenfriend.

Cooperating Units: Clinical Biochemistry Laboratory

Man Years
Total: .50
Professional: .25
Other: .25

Patient Days: 200

Project Description: Previous work in this section on serotonin and the catecholamines has stimulated our interest in their precursor amino acids, alternate pathways of metabolism, and in other amino acids. A sufficient amount of work has been done to warrant a separate project description.

Hydroxyproline (OPR) - This substance is a constituent of connective tissue being found uniquely in the collagen-protein of the body. During the past year measurements of blood levels and urinary excretion of OPR have been made in 71 individuals, with emphasis on patients with hereditary disorders such as Marfans Syndrome. Blood levels have been found to be quite variable (av = 1 μgm/cc) whereas daily urinary excretion is fairly constant in the normal adult usually being 15-23 mgm/day. Sporadic high values have been found in various conditions, this being most consistent in the case of Marfans Syndrome wherein 7 of 9 patients had values in the range of 53-120 mgm/day.

Tolerance Tests - Ziff (1956) had shown that the administration of 4.0 gm. OPR orally did not result in any increase of the urinary excretion above control values, but that if the same amount of OPR was given in the form of gelatin, the excretion rose. It was suggested that a bound (peptide) form of the amino acid was required for excretion. We have shown that the blood level of free OPR rises in a similar fashion after OPR or gelatin orally. However, excretion in the urine was found simply to depend on the presence of other amino acids since OPR plus casein resulted in increased OPR excretion. Various amino acids have been given in combination with OPR to ascertain which amino acid might be responsible for the excretion following gelatin and OPR-casein. To date, only glutamic acid has been found to induce OPR excretion..... Two cases of Ehler-Danlos Syndrome and several cases of Marfans Syndrome have behaved abnormally in that significant increases in urinary OPR followed its oral administration.

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The Indoleacetic Acid (IAA) Pathway of Tryptophan Metabolism. Study of IAA (an Auxin in plants) in man has been limited by the lack of satisfactory means of assay. A simple chemical method of measurement in urine and tissue was developed, which is dependent on extraction into chloroform and a specific color reaction with xanthydrol. Normal daily excretion was found to be 4.0 to 18.0 mgm/day. Survey of a variety of clinical conditions revealed elevated levels in 5 patients with apparently unrelated clinical conditions, diabetes, idiopathic sprue, cerebellar ataxis and amyotrophic lateral sclerosis. Gut sterilization with neomycin and tetracycline (0.5 gm q6h) reduced the IAA by 30% in normals and 70% in a patient excreting 200 mgm/day, suggesting that some of the urinary IAA was derived from extra-intestinal metabolism of tryptophan. Conversion of tryptophan to IAA in tissues was confirmed by in vitro experiments (see report of L.C.B. for details). Tryptophan loading in man resulted in a marked increase in IAA excretion for several hours while no increase in 5HIAA excretion could be observed. This suggests that measurements of IAA excretion might be used as an indicator to detect abnormalities in tryptophan metabolism.

O-hydroxylation of phenylalanine in patients with phenylketonuria. The presence of O-hydroxyphenylacetic acid in the urine was confirmed and C14 labeling shown to occur from administered C14 phenylalanine in two patients with phenylpyruvic oligophrenia. Attempts to find a possible intermediate which would be of physiologic interest, namely o-tyramine, were unsuccessful.

Hydroxyproline - Study in more detail factors governing the urinary excretion of this amino acid.

Indoleacetic Acid - Ascertain whether tryptamine (a physiologically active compound) might be an intermediate between tryptophan and IAA.

Part B included Yes [ ] No [X]

Form No. ORP-2
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Serial No. NHI-205
1. GMET
2. Exp. Therapeutics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Possible Role of Serotonin in Pulmonary Embolus in the Dog and its Implications in Humans.

Principal Investigator: T. P. Waalkes, M.D., Ph. D.

Other Investigators: Richard Sanders, M.D. and Harriet Coburn.

Cooperating Units: None

Man Years
Total: .40
Professional: .25
Other: .15

Patient Days: None

Project Description: Previous work had indicated that serotonin might be a toxic factor during pulmonary embolus in the cat, an animal which has a relatively large amount of serotonin in its blood. The dog as compared to the human has about the same amount of serotonin in its blood. This amount is much less than that found in the blood of cats.

Initial studies have shown that serotonin is probably not a toxic factor during pulmonary embolus in the dog.

To complete the above studies, and by analogy compare the results to what may also happen in human pulmonary embolization in regards to serotonin.

Part B Included Yes [ ] No [X]

Form OER-2
Oct. 1957

Serial No. NHI-206 (C)
1. GMBT
2. Exp. Therapeutics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on Vasoactive Substances.

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

Other Investigators: T. Phillip Waalkes, M.D., Ph. D.; J. Richard Crout, M.D.; John D. Davidson, M. D.; B. J. Haverback, M.D.; Luther L. Terry, M.D.; H. Weissbach, Ph. D.; and S. Udenfriend, Ph. D.; C. Garcia and W. King.

Cooperating Units: Laboratory of Clinical Biochemistry.

Man Years
Total: 2.5
Professional: 1.50
Other: 1.0

Patient Days: 1100

Project Description: This project was begun in 1953 as one approach to the problem of hypertension and has led to many unexpected avenues of research.

Carcinoid Syndrome - We continue to receive numerous specimens of urine for 5-HIAA and tissue samples for serotonin assay from elsewhere. These assays, though a burden on our laboratory are being done to promote good will and maintain close connection with developments in the field. We are continuing to admit patients with this condition for evaluation and whenever possible the patients are then transferred to the care of Dr. Paul Condit (NCI) for administration of anti-neoplastic drugs. We have now shown conclusively that there is no significant difference in pulmonary A-V plasma serotonin to account for predominant right-heart disease. A possible error due to release of serotonin from platelets during preparative procedures was overcome by first eliminating platelet serotonin in vivo by the reserpine therapy. Administration of a single dose of iproniazid (Marsilid), a drug which has been shown to block serotonin metabolism in vivo, resulted in marked aggravation of symptomatology in one patient. The fact that the intestine is the major depot of serotonin normally was shown dramatically by our inability to find any significant amount of serotonin in the blood or 5-HIAA in the urine of a patient who had been subjected to removal of all but 2½ feet of intestine.

5-OH-Tryptophan (5HTP), the Precursor of Serotonin - Additional infusions of 25 mgm doses have been done in man, and resulting stimulation of small intestinal motility clearly shown.

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Catecholamines - Here again, numerous specimens have been received from elsewhere reflecting our prominence in this field. Rate of nor-epinephrine turnover using the C^{14} label was calculated in another patient with pheochromocytoma and again found to be a matter of hours, in contrast to the turnover in adrenal gland which is a matter of days. Since Armstrong's report (Fed. Proc. 1957) that a major urinary metabolite of epinephrine and nor-epinephrine is 3-methoxy-4-OH-mandelic acid (VMA) several groups at NIH, including ourselves, have been working on various phases of O-methylation of catecholamines. Both O-methoxy epinephrine (ME) and O-methoxy nor-epinephrine (MN) have been found in rat urine by Axelrod (NIMH) and in tissue homogenates by Axelrod (NIMH) and Leeper (LCB) after exhibition of the corresponding precursor amine. Further details are in reports from L.C.B. More recently MN has been found in animal brain (Axelrod and Shore L.C.P.) Four compounds were made available for chemical and pharmacologic study; VMA by Dr. M. Fish (LCNP), ME, MN and O-CH_3 - dopamine (MD) by Dr. B. Witkop (NIAMD). All means of assay conceivable to us were attempted in order to measure urinary VMA simply, without success. Such a measurement would be extremely useful in the diagnosis of pheochromocytoma and in learning more about the metabolism of catecholamines in various clinical conditions but to date a simple means of separating VMA from numerous other phenolic acids in urine has been impossible. Reasoning that the methoxycatecholamines would be rapidly converted to the corresponding acids and hence, not excreted to any great extent in the urine, extensive study of pheochromocytoma tumor tissue was undertaken. Although the results are not yet conclusive, we believe we have shown the presence of MN and MD, a third substance may be either ME or methoxydopa. All of this work is being done on material received from elsewhere. Both MN and ME were found to be extremely weak pressor substances in anesthetized dogs, 5 mgm producing an effect equivalent to 5 ugm of norepinephrine. Hence, physiologically, O-methylation may be considered as a means of inactivation.

Another phase of this investigation is to evaluate and modify the present published methods for the quantitative fluorometric assay of epinephrine (E) and norepinephrine (NE) in plasma and urine in an effort to obtain a reproducible, accurate assay procedure for research purposes. Previous work by others in this laboratory has shown the plasma method of Weil-Malherbe and Bone (ethylene-diamine condensation) to be accurate in determining total plasma catecholamines, but the procedure is tricky technically and the separation of NE from E is believed to be somewhat less than ideal. The present urine method in use at this laboratory is based on the differential oxidation of NE and E at pH 3.5 and pH 6.0, using iodine as the oxidizing agent.

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This method is felt to be satisfactory for the diagnosis of pheochromocytoma, but the presence of a number of interfering substances limit its usefulness in detecting small changes in catecholamine excretion.

Analysis of standard solutions and of the fluorometric curves obtained showed the differential pH oxidation procedure to be equally sensitive to the ethylene-diamine condensation in detecting small concentrations of NE and E and to be somewhat superior in separating NE from E at the low concentration found in plasma. A differential pH oxidation method using ferricyanide as the oxidizing agent (modified from von Euler and Floding) was consequently devised and adapted to blood plasma. This procedure is technically more simple than the commonly used ethylene-diamine technique and is giving normal values within the known normal range (2-7 γ% plasma). In the only clinical application to date, plasma catechol assays were done on samples obtained from various levels within the vena cava by cardiac catheterization on a patient with known pheochromocytoma (patient hospitalized at the National Naval Medical Center).

These assays proved helpful in localizing the tumor pre-operatively. Preliminary studies have shown that this assay can be applied to small volumes of urine (10-20 ml) but specificity is not yet ideal because of interfering substances. Future work is anticipated in improving the specificity of the urine assay and establishing the accuracy and intrinsic error of both the blood and urine methods. It is hoped that the method can then be applied to a study of catecholamine relationships in various disease states.

Mast Cells - (Project started in January 1957 and is near completion) On the basis of studies in rats, Benditt et all and Parrett and Uesh had implied serotonin-release from mast cells in mediation of the inflammatory response to various agents. Presence of heparin and histamine in mast cells of various species was known previously. The following mast-cell preparations became available for study in January 1957: transplantable mast cell tumors of the mouse (courtesy Drs. T. B. Dunn and M. Potter, NCI, and the dog (Dr. C. S. Lombard, NCI) and skin biopsy and urine specimens (courtesy Major M. J. Daniels Walter Reed) on a patient with urticaria pigmentosa (a condition characterized by dense accumulations of mast cells under the skin). Subsequently, tissue and urine specimens became available on two additional patients with "mast cell disease". The rodent mast cell tumor contained large amounts of both serotonin and histamine, whe[illegible] human mast cells contained only histamine. Hence, a species differen[illegible]

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was clearly shown and cautions against invoking serotonin-release from mast cells in human physiologic mechanisms and raises the question of the suitability of rodents for screening of antiinflammatory drugs. With regard to serotonin metabolism, the tumor bearing mice resembled patients with carcinoid. An extensive study of the biochemistry of the mouse tumor and the effect of various drugs and its serotonin and histamine content has been done. Of some interest was a specific decrease in serotonin level with cortisone therapy. However, administration of cortisone to normal rabbits (25 mgm/kgm x 2 wk) did not affect tissue levels of serotonin. Use of this tumor for other studies, such as heparin synthesis by Dr. Ed Korn (NHI), tissue culture for *in vitro* production of serotonin, etc. was made possible by our work.

Direction of current research - Carcinoid Syndrome - None at the moment, 5-OH-Tryptophan - Study effects on intestinal motility after oral administration, praticularly in patients with constipation due to antihypertensive drugs, catecholamines - study *in vivo* formation of norepinephrine from administered precursors, compare normals and hypertensives, study effect of various drugs on adrenal vein amines in man; and mast cells - indefinite.

Part B included Yes [X] No [ ]

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Part B: Honors, Awards, and Publications.

Sjoerdsma, Albert; Waalkes, T. P.; Weissbach, H.; Serotonin and Histamine in Mast Cells, Science 125: 1202-1203, 1957.

Sjoerdsma, Albert; Weissbach, H.; Terry, Luther L.; Udenfriend, S.; Further Observations on Patients with Malignant Carcinoid, Am. J. of Med. 23: 5-15, 1957.

Sjoerdsma, Albert; Terry, Luther L., and Udenfriend, S.; Malignant Carcinoid, a New Metabolic Disorder, AMA Arch. Int. Med 6: 1009-1112, 1957

Carcinoid Syndrome (Carcinoidois) Textbook of Medicine (Cecil-Loeb) In press

Davidson, J. D.; Sjoerdsma, Albert; Loomis, L.; Udenfriend, S.; Studies with the Serotonin Precursor, 5 Hydroxytryptophan, in Man and Experimental Animals; J. Clin. Invest. In press.

Form No. ORP-2
Oct. 1957

Serial No. NHI-207
1. CMET
2. Exp. Therapeutics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Serotonin (and Histamine) in Lung.

Principal Investigator: T. Phillip Waalkes, M.D.

Other Investigators: H. Weissbach, Ph. D. and S. Udenfriend, Ph. D.

Cooperating Units: Laboratory of Clinical Biochemistry

Man Years
Total: .40
Professional: .25
Other: .15

Patient Days: None

Project Description: Serotonin has been found to be present in the lungs of a variety of laboratory animals. Since the lungs play an important part in anaphylactic reactions, the presence of serotonin could be of importance from this standpoint.

It appears, however, that the amount of serotonin in human lung is very small, and particularly so compared to the amount of histamine.

More analyses must be done on human lungs and lungs from other laboratory animals.

Serial No. NHI-207

[redacted]
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Part B: Honors, Awards, and Publications

Weissbach, H.; Waalkes, T. P.; and Udenfriend, S.: The Presence of Serotonin in Lung and Its Implication in the Anaphylactic Reaction. Science, 125, 235-236 (1957).

Form No. [illegible]
Oct. 1957

Serial No. NHI-203 (c)
1. GMBT
2. Exp. Therapeutics
3. Bethesda, Md.

PHS-[illegible]
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Action and Metabolism of Drugs

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

Other Investigators: Louis Gillespie, M.D., Luther L. Terry, M.D.
C. Garcia and W. King

Cooperating Units: None

Man Years
Total: 1.00
Professional: .50
Other: .50

Patient Days: 100

Project Description: Little in the way of original work has been done in this area during the past year. In the therapy of hypertension, mecamylamine (Inversive) has proved to be a superior "blocking agent" in our experience and we no longer use the quaternary ammonium compounds. Preliminary studies with ipronidazid (Marsilid) do not confirm favorable response in hypertension as claimed by others. Measuring various substances during therapy has so far led only to the findings of increased blood serotonin and decreased urinary 5HIAA, confirming animal experiments.

Explore possible techniques of altering serotonin and norepinephrine metabolism in man and relate to effects on blood pressure.

Part B Included Yes [X] No [ ]

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Davidson, John D.; Terry, Luther L. and Sjoerdsma, Albert
Action and Metabolism of Chlorpromazine Sulfoxide in Man,
Journal of Pharmacology and Experimental Therapeutics, Vol. 121,
No. 1, 8-12, September 1957.

Form No. CRP-2
Oct. 1957

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on Anaphylaxis

Principal Investigator: T. Phillip Waalkes, M.D., Ph. D.

Other Investigators: H. Weissbach, Ph. D., J. Bozicevich, M.S. S. Udenfriend, Ph. D. and H. Coburn.

Cooperating Units: Laboratory of Immunology, NIAID

Man Years
Total: .50
Professional: 25
Other: .25

Patient Days: None

Project Description: Serotonin, as well as histamine, is released into the blood plasma during anaphylaxis in the rabbit. Initial studies indicate that most of this serotonin is liberated from the blood platelets. Further studies show, however, that the lung level for serotonin is markedly elevated immediately after the injection of an antigen into a sensitized rabbit. This increase is probably due to the trapping of agglutinated platelets.

Further work is planned to determine the role of serotonin and histamine during anaphylaxis and hypersensitivity reactions. Blood and tissue studies are in progress both in vivo in animals and in vitro (with blood) in humans. The studies with rabbits are continuing but also have now been extended to other laboratory animals. In addition the role of platelets in hypersensitivity is being investigated.

Human S. Included Yes [X] No [ ]

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Serial No. NH(-P[illegible]

PHS-NIH
Individual Project Report
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Part B: Honors, Awards and Publications

Waalkes, T. P.; Weissbach, H.; Bozicevich, J.; and Udenfriend, S. Serotonin and Histamine Release During Anaphylaxis in the Rabbit. J. Clin. Inves., 36; 1115-1120, (1957).

Waalkes, T.P.; Weissbach, H.; Bozicevich, J.; and Udenfriend, S. Further Studies on Release of Serotonin and Histamine During Anaphyl[illegible] in the Rabbit. Proc. Soc., Exp. Biol. and Med., 95: 5-15 (1957)

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A:

Project Title: Studies on Serotonin and Histamine in Mast Cell Tumors

Principal Investigator: T. P. Waalkes, M. D., Ph.D

Other Investigators: A. Sjoerdsma, M. D., Ph.D., and H. Weissbach, Ph. D.

Cooperating Units: Laboratory of Clinical Biochemistry

Man Years:
Total: .40
Professional: .25
Other: .15

Patient Days:
None

Project Description:

These studies have been carried out largely on the DBA strain of mice harboring either an ascitic type or a subcutaneous type of transplantable mast cell tumor. Analyses have shown that both serotonin and histamine are present in the tumors in large amounts and these amounts increase with age. An expressive quantity of 5HIAA is found in the urine of these mice; histamine studies on urine have not been done as yet.

In addition, tissue from humans with urticaria pigmentosa, a mast cell lesion, has, on analysis been found to have little serotonin but a large amount of histamine. Carcinoid tumors, on the other hand, have a large amount of serotonin, but little histamine.

It appears that mast cells in humans have little serotonin though a large amount of histamine.

Further studies are in progress on the effect of a variety of drugs on the serotonin and histamine content of the mice mast cell tumors. In addition, precursors of serotonin and histamine are being studied in these mice.

Part B included Yes [ ] No [X]

Form No. CRP-2
Oct. 1957

Serial No. NHI-211
1. GIET
2. Experimental Therapeutics
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A:

Project Title: The Analysis of Serotonin and Histamine in Blood and Tissues.

Principal Investigator: T. P. Waalkes, M.D.

Other Investigators: M. Weissbach, Ph.D. and S. Udenfriend, Ph.D.

Cooperating Units: Laboratory of Clinical Biochemistry

Man Years:
Total: .40
Professional: .25
Other: .15

Patient Days:
None

Project Description:

In connection with the work on anaphylaxis and mast cell tumor mice, sensitive methods of assay for both serotonin and histamine were needed. By the use of new agents and, also by modifications of existing procedures, these methods were developed and so adapted that both serotonin and histamine could be analyzed in the same tissue aliquot.

The above methods will be utilized in any further research involving serotonin and/or histamine.

Part B included Yes [X] No [ ]

Serial No. NHI-211

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

The Serotonin and Histamine Methods are at present in press in the J. of Biol. Chem.

Form CRC-3
Oct. 1957

Serial No. NHZ-
1. CMC
2. Exp. Therapeutics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Histamine Metabolites

Principal Investigator: T. Phillip Waalkes, M.D., Ph. D.

Other Investigators: Helen Price

Cooperating Units: None

Man Years
Total: .40
Professional: .25
Other: .15

Patient Days: None

Project Description: Recent radioactivity studies have shown a new histamine derivative found in human and animal urine. This metabolite is thought to indicate the main route of histamine metabolism, since initial work has shown this compound to be present in much greater quantity than histamine itself. Other histamine metabolites have also been found.

Studies have been devoted largely to the developing of methods of identification and analysis for these histamine derivatives. In several instances, the compounds themselves are not available and the syntheses of these compounds is now in progress.

The present plan is to make a thorough study of histamine metabolism in humans and animals.

Part B included: Yes [ ] No [X]

Form No. ORP-
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[redacted]

[redacted]

Individual Project Report
Calendar Year [redacted]

Part A.

Project Title: Amino Acid Studies in Humans.

Principal Investigator: T. P. Waalkes, M.D., Ph. D.

Other Investigators: S. Udenfriend, Ph. D.

Cooperating Units: Laboratory of Clinical Biochemistry.

Man Years:
Total: .65
Professional: .50
Other: .15

Patient Days: None

Project Description: Studies had been initiated on plasma levels of tryptophane and tyrosine in patients. Initial observations indicated that tryptophane levels were low in the plasma of patients with rheumatoid arthritis while they were taking steroids. No further work has been done on this.

A new fluorometric method for tyrosine in plasma and tissues was worked out. No work on this project is contemplated in the near future.

Part B included: Yes [X] No [ ]

Serial No. NHT-213

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B. Honors, Awards and Publications.

Waalkes, T. Phillip and Udenfriend, S.; A Fluorometric Method for Tyrosine Assay in Plasma and Tissues. J. of Lab. and Exp. Med. In press.

1 [illegible]
2 Exp. Therapeutics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Effect of Indole Compounds on the Gastrointestinal Tract.

Principal Investigator: Bernard J. Haverback, M.D.

Other Investigators: Albert Sjoerdsma, M.D., Ph. D., Luther L. Terry, M.D., C. Garcia and W. King.

Cooperating Units: None

Man Years:
Total: .40
Professional: .25
Other: .15

Patient Days: 500

Project Description: Studies of serotonin metabolism in the carcinoid syndrome have led to interest in this and other compounds with the indole nucleus. It has been considered that further elucidation of the effects of these substances on the G.I. tract and their interrelationship with other chemical substances is important to a proper understanding of normal physiology and the alterations seen with certain diseases and during the administration of some therapeutic agents.

Since the principal investigator was with the National Heart Institute only during the first six months of the year studies were directed largely toward correlating some of the previous observations and to preparing the material for publication.

Previous studies have shown that serotonin is a potent stimulus to gastrointestinal motility and an inhibitor of gastric secretion. Administration of 5-hydroxytryptophan (5-HTP) in animals increased both tissue and circulating serotonin and inhibited gastric secretion induced by insulin hypoglycemia and by urecholine. However, it did not inhibit secretion stimulated by reserpine or histamine. When given in large doses to rats 5-HTP produced gastric mucosal ulceration.

The infusion of 25 mg doses of 5HTP in man showed stimulation of small intestinal motility. Iproniazid (Marsilid) a drug which blocks the metabolism of serotonin *in vivo*, showed a marked aggravation of general symptomatology and particularly of intestinal cramps and number of stools. An interesting observation was made in a patient who had surgical removal of all but 2½ feet of the intestinal tract no serotonin could be demonstrated in blood or 5-HIAA in the urine.

The large part of this program has been completed and with the loss of the staff of the principal investigator, this program as such has been terminated. However, certain aspects of the program will be continued in our studies of vasoactive substances. Further observations will be made on the effect of orally administered 5HTP in patients with consti-

Serial No. [illegible]

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Haverback, Bernard J. and Bogdanski; Gastric Mucosal Erosion in the Dog Following Administration of the Serotonin Precursor, 5-Hydroxytryptophan. Proc. Soc. Exp. Biol and Med. 95: 392-393, 1957.

Haverback, Bernard J.; Hogben, C.A.M.; Moran, M.C.; and Terry, L. L. Effect of Serotonin and Related Compounds on Gastric Secretion and [illegible] in the Dog. Gastroenterology 32: 1058-1065, June 1957.

Form No. ORP-2
Oct. 1957

Series A-[illegible] NHI-[illegible]
1. [illegible]
2. Exp. Therapeutics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Release of Histamine by Reserpine.

Principal Investigator: T. P. Waalkes, M.D., Ph. D.

Other Investigators: H. Weissbach, Ph. D.

Cooperating Units: Laboratory of Clinical Biochemistry

Man Years
Total: .40
Professional .25
Other .15

Patient Days: None

Project Description: In vivo experiments have shown that reserpi[illegible] liberates histamine from rabbit platelets. Whereas most of the [illegible] histamine is released in the rabbit and other laboratory animals.

In vitro studies are in progress to elucidate the mechanism of [illegible] histamine and serotonin release from platelets. Studies in human[illegible] have not been started, although planned for the future.

Part B included: Yes [X] No [ ]

Serial No. NHI-215

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B. Honors, Awards, and Publications

Waalkes, T. P., Weissbach, H.; In Vivo Release of Histamine from Rabbit Blood by Reserpine; Proc. Soc. Exp. Biol. & Med. 93: 394-396 (1956)

Form No. CRP-2
Oct. 1957

Serial No. NHI-216 (c)
1. GMET
2. Cardiodynamics
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A:

Project Title: Studies in Valvular Insufficiency; The Effect of Pharmacologic and Exercise Stress on Aortic and Valvular Insufficiency as Studied by the Left Heart Catheterization Technique.

Principal Investigators: Herbert L. Tanenbaum, Samuel M. Fox, 3rd Andrew G. Morrow, Kingsley Lawrance

Other Investigators: Frederick A. Bullock, Estelle R. Cohen, Katherine A. Hirschfield, Clara V. King

Cooperating Units: Surgical Branch

Man Years (calendar year 1957):
Total: .30
Professional: .15
Other: .15

Patient Days (calendar year 1957):
100 ± . 10 patients

Project Description:

Techniques of evaluating the dye dilution curve have been under study as well as the pulse contour of left atrial pressures before, during, and after the administration of Norepinephrine, Vasoxyl and exercise stress.

The anticipated course of the study will include the later use of the velocity catheter.

Human Subjects Included Yes [ ] No [X]

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Serial No. NHI-217 (c)
1. CMET
2. Cardiodynamics
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Architectural Aspects of Diseases of the Heart

Principal Investigator: Robert P. Grant

Other Investigators: Clara V. King, Margaret Austin

Cooperating Units: None

Man Years (calendar year 1957):
Total: .40
Professional: .20
Other: .20

Patient Days (calendar year 1957): 80 +

Project Description:

A few years ago the study of the 3-dimensional architectonics of the heart in various forms of acquired heart disease was undertaken, culminating in two published reports on this subject. With the accumulating volume of pathologic material, it is now possible to resume this study with principal attention on congenital heart disease.

a) One of the serious shortcomings of cardiac pathology in the past has been its dependence on uni-dimensional, "landmark" morphologic description. Considerable time has been spent in testing other approaches to defining quantitatively the architectural changes in congenital heart disease. Principal emphasis so far has been placed on the techniques of topology. This permits novel schematization of cardiac morphology. The cardiovascular system proves to be an elaboration of the simplest surface, the Moebius ring. Mathematical techniques for defining the pulmonary artery - aorta torsion (so important in transposition and truncus problems) are under similar study and experimentation. Transformational mathematical techniques seem to have merit for this purpose.

b) Three cases of what may generally be called dextroversion of the heart have been studied clinically and one pathologically. In addition, one case of isolated ventricular inversion has come to post mortem study. Analysis of these cases is in progress, with study of the nature of heterotaxy in other regions of the body in these cases and leading to critical evaluation of the problems of laterality, especially as applied to cardiac embryology. It is planned also to do

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Project Description (cont.):

cardiac musculature dissections in these cases- a type of study of congenital heart disease which has not previously been pursued. In addition, by wire implants in the fibrous valvular rings in hearts with rotational and transpositional defects, then X-rayed, objective measurements of the lie of certain structures can be determined. This has already been done in 6 hearts and when the methods of wiring and also of musculature dissection are perfected, the great wealth of pathologic material at A.F.I.P. will be studied with the generous permission of Dr. William Manion.

c) There has so far been absolutely no work done on the nature of cardiac hypertrophy, and, incredibly enough, it remains unknown whether the hypertrophied fibre is, in fact, stronger than the "normal" fibre or what the hormonal and metabolic factors are underlying the specialized type of growth we call hypertrophy. With availability of animal experimentation facilities it is planned to start studies on the extremely important and, so far, totally neglected subjects of the nature and mechanism and architectonics of experimental cardiac hypertrophy. It is hoped in time to extend these studies to biochemical methods in clinical and experimental material for measuring the component of fibrosis that takes place with advancing cardiac hypertrophy. It is not generally appreciated that the "growth" aspect of cardiac hypertrophy has no parallel in its extensiveness in any other organ or muscle system, normal or abnormal.

Part B included Yes [ ] No [X]

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Serial No. NHI-218 (c)
1. GMET
2. Cardiodynamics
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A:

Project Title: The More Precise Quantitation of the Primary Lesion of Chronic Diffuse Pulmonary Emphysema.

Principal Investigators: Robert Hyatt, Donald P. Schilder, Donald L. Fry

Other Investigators: Alexander Mallos, Jean Caha, Raymond Kelly

Cooperating Units: None

Man Years (calendar year 1957):
Total: 1.01
Professional: .66
Other: .35

Patient Days (calendar year 1957):
600

Project Description:

From theoretical considerations and data previously published the "primary" lesion of emphysema is at the bronchiolar level. Theoretically at every point in lung inflation there must exist a maximum expiratory flow beyond which a subject cannot go. This maximum flow depends on the bronchiolar compliance, resting radius, and the lung compliance. Oscilloscopic plots of the maximum expiratory flow versus volume have been obtained in normals and in emphysematous subjects and have been found to be extremely reproducible. There are ten to thirty fold differences in the ordinate values between the two groups. Mathematical analyses of this curve with the lung compliance curve should yield values for bronchiolar compliance and dimensions.

Current research is directed toward quantitation of bronchiolar compliance and dimensions.

Part B included Yes [ ] No [X]

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Project Description (cont.):

2) To establish the magnitude of stress necessary to produce lesions a monkey preparation will be used. The intact thoracic cavity with lungs inflated will be placed in a pressure chamber where they can be ventilated at various frequencies and amplitude. The air ways will be liquid filled to exaggerate the flow stresses. After various prescribed stress maneuvers the lung will be fixed and examined microscopically to determine the distribution, orientation, and magnitude of the tears. This project is just underway and no results have been obtained. The pressure chamber has been built and successfully tested.

3) The nature of the bronchiolar abnormality is being studied radiographically in excised animal lungs. A fine contrast medium (micropaque) is being blown in to outline the bronchioles. Dimensions and compliance measurements are then taken at various degrees of inflation. The bronchiolar system has been found to be extremely compliant as compared to the upper airways, and total collapse occurs with very small positive transmural pressures. Emphysematous lungs have not yet been studied. This project began June 1967 and is continuing.

Persuance of the objectives as outlined above.

Part B included Yes [X] No [ ]

Serial No. NHI-218 (a)

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Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

McCall, C. B., Hyatt, R. E., Noble, F. W. and Fry, D. L.:
Harmonic Content of Certain Respiratory Flow Phenomena of Normal
Individuals. J. Appl. Phys. 10:215-218, 1957.

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Serial No. NHI-219
1. GMET
2. Cardiodynamics
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A:

Project Title: The Development of Basic Instrumentation and the Evaluation of Existing Instruments for Physiological Application in the Field of Fluid (gas and liquid) Flow and Pressure Measurement.

Principal Investigators: Donald L. Fry, Frank W. Noble, Robert E. Hyatt
Charles B. McCall

Other Investigators: Alexander J. Mallos, Jean Caha, Alfred Casper

Cooperating Units: Laboratory of Technical Development

Man Years (calendar year 1957):
Total: .90
Professional: .55
Other: .35

Patient Days (calendar year 1957):
None

Project Description:

The commercially available pressure measuring systems have been carefully evaluated as to baseline stability, gain stability, linearity, dynamic accuracy and acceleration errors. Systems heretofore thought inadequate were found superior for certain applications to those classically used. With the exception of the Gauer-Wetterer microgage all systems were found to have a disappointingly large acceleration error when used with a liquid pressure transmitting system such as a catheter. The results of this study indicate the urgent need for the development of an improved, commercially available microgage of this type. This project began in June 1955 and ended in January 1957.

A catheter tip method of instantaneously and continuously computing the aortic blood velocity was developed. This consisted of three parts: (1) development of a theoretical structure for a working hypothesis, (2) developing and evaluating a liquid differential pressure measuring scheme, (3) developing and evaluating an electronic analogue computer to carry out the instantaneous computations indicated by the theory. A working system has been applied to numerous animals and gives reasonable results; however, before this system is used in human subjects many critical studies need to be done. This project began in January 1956 and is yet in progress.

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Calendar Year 1957

Project Description (cont.):

A new type of respiratory flow meter was developed and it with existing types was evaluated as to stability, linearity, and dynamic accuracy. The new type of flowmeter was found to have two major advantages (low dead space, and immunity to condensation effects) over existing types. After much use one disadvantage has been noted. An unexplained transient error has been noted which is not reflected in its dynamic response curve. This project began in July 1956 and ended in March 1957.

Project finished. Commercial companies who will work on the improvement and production of a microgage are being sought.

A fluid tunnel analogous to a wind tunnel in aerodynamics is being designed and will be built so that known flow functions with known viscosities and densities can be studied by the various flow measuring techniques. This is the only way to firmly establish the validity of the catheter tip flow measuring device. Not only will this flow tunnel have this application, but many others related to the more accurate definition of the flow patterns and their controlling parameters in the vascular tree.

Commercial companies are being encouraged to develop better respiratory flow and volume measuring devices.

Publ. is included [X] yes [ ] no

Serial No. NHI-219

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Fry, D. L., Noble, F. W., and Mallos, A. J.: An Evaluation of Modern Pressure Recording Systems. Circulation Research 5:40-46, 1957.

Fry, D. L., Hyatt, R. E., McCall, C. B., and Mallos, A. J.: Evaluation of Three Types of Respiratory Flowmeters. J. Appl. Phys. 10:210-214, 1957.

Fry, D. L., Noble, F. W., and Mallos, A. J.: An Electric Device for Instantaneous and Continuous Computation of Aortic Blood Velocity. Circulation Research 5:75-78, 1957.

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Oct. 1957

Serial No. NHI-220 (c)
1. GMET
2. Cardiodynamics
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A:

Project Title: Study of the Relationship of Pulmonary Function to the Parameters of Cardiovascular Function.

Principal Investigators: Donald P. Schilder, Robert E. Hyatt, Samuel M. Fox, and Donald L. Fry

Other Investigators: Alexander Mallos, Jean Caha, and Raymond Kelly

Cooperating Units: None

Man Years (calendar year 1957):
Total: 1.06
Professional: .71
Other: .35

Patient Days (calendar year 1957):
600 days, 10 patients

Project Description:

Most of the progress has been in instrumentation, however some preliminary physiological data has also been collected.

1) Instrumentation

The parameters of lung function are the intrathoracic pressure measured by the intraesophageal balloon, the respiratory flow measured by one of several types of flowmeters which have been developed, improved or evaluated in this lab, and respired volume. Techniques for measuring the first two parameters have been successfully developed over the past three years. A technique for the measurement of the instantaneous lung volume has been less tractable. The problem consists of instantaneously replacing the oxygen in the spirometer circuit as it is metabolized. The scheme that has showed most promise has been the use of a thermal conductivity cell to sense the N_2:O_2 ratio. As O_2 is burned this ratio rises causing an electrical unbalance of the cell which signals a servo-amplifier to replace the O_2 deficit. Formidable problems of mixing appear to have been overcome and the remaining problem consists of increasing the sensitivity of the conductivity cell.

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Calendar Year 1957

Project Description (cont.):

2) Physiological

An alternate less ideal method of sensing the lung volume has been employed while the above system is being perfected so that physiological studies may be started. Normal and diseased subjects have been studied with the following results. Alterations of compliance and flow resistance confirm data previously published by members of this lab. New findings of a preliminary nature indicate rather striking acute changes in compliance with Valsalva and Mueller maneuvers. These changes are functions of the position of the chest and volume of the lung. Cardiacs and emphysemas show more marked changes than do normals. This further supports the working hypothesis that pulmonary vascular transmural pressure plays an important role in the production of lung compliance.

Current research consists of pursuing these findings and defining specifically how the vascular pressure operates to produce this effect. It is hoped that quantitation of this effect will lead to an indirect measure of the pulmonary artery, capillary, and vein pressure.

Part B included Yes [ ] No [X]

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Serial No. NHI-221
1. GMET
2. Cardiodynamics
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Use of Indicator-Dilution Curves from the Left Heart and Aorta in the Localization of Left-to-Right Shunts in Patients.

Principal Investigators: Andrew G. Morrow, Eugene Braunwald, Herbert L. Tanenbaum

Other Investigators: Estelle R. Cohen, Fred Bullock

Cooperating Units: Surgery Branch

Man Years (calendar year 1957):
Total: 3
Professional: 2
Other: 1

Patient Days (calendar year 1957):
None

Project Description:

The precise localization of the origin of the left-to-right shunt may be difficult by determination of the oxygen differences from the cardiac chambers even when an inert gas, such as nitrous oxide, is used. These techniques are not applicable in the detection, for example, of valvular insufficiency in association with the left-to-right shunts. The techniques of left heart catheterization and aortic catheterization have made possible the injection of indicator dye into the left atrium and left ventricle and various sites in the aorta. Indicator dilution curves have been obtained from these sites and their value assessed in the localization of left-to-right shunts.

More than 100 patients have now been studied. In the absence of a left-to-right shunt, or valvular regurgitation, a left heart aortic dye injection results in a primary curve with a sharp, rapid ascent and descent. When injection is made proximal to the site of the shunt, a portion of the dye passes through the pulmonary circulation and interrupts the descending limb of the dye curve with the secondary peak. By selective injections at various locations the origin of the left-to-right shunt can be detected. Similarly, valvular insufficiency results in a prolonged, but smooth, descending limb of the dye curve and by selective injection, valvular insufficiency may also be localized. By combining dye injections with the technique of cardiac catheterization and the use

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Calendar Year 1957

Project Description (cont.):

of the nitrous oxide test, we are presented with numerous methods for localizing the shunt. A problem has presented itself in the detection of small shunts and the differentiation of them from valvular insufficiency. The curves are presently being recalibrated on semi-logarithmic paper and the slopes of the ascending and descending limbs being compared. It is believed that this will result in more precise methods of analysis of the results in curves. The investigations are being continued in an increasing number of patients.

Yes [X] No [ ]

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Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Grant, R. P., Sanders, R. J., Morrow, A. G., and Braunwald, E.: Symposium on Diagnostic Methods in the Study of Left-to-Right Shunts. Circulation 16:791-802, November 1957.

Braunwald, E., Tanenbaum, H. L., and Morrow, A. G.: Localization of Left-to-Right Cardiac Shunts by Dye Dilution Curves following Injections into the Left Heart and Aorta. In press. Am. J. Med.

Baker, R. R., Braunwald, E., Tanenbaum, H. L., and Morrow, A. G.: The Localization of Left-to-Right Cardiac Shunts. An experimental study of indicator dilution curves following left heart and aortic injections. In press. Annals of Surgery.

Wood, J. C., Conrad, M. E., Jr., and Morrow, A. G.: Partial Anomalous Venous Connection. A case report illustrating diagnostic techniques. Am. Heart J., 54:422-428, September 1957.

Braunwald, E., Tanenbaum, H. L., and Morrow, A. G.: Dye-dilution Curves from Left Heart and Aorta for Localization of Left-to-Right Shunts and Detection of Valvular Insufficiency. Proc. of the Soc. for Exper. Bio. and Med., 94:510-512, 1957.

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Oct. 1957

Serial No. NHI-222
1. GMET
2. Cardiodynamics
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Experimental Proof of the Value of Indicator Dilution Curves in the Localization of Left-to-Right Circulatory Shunts.

Principal Investigators: R. Robinson Baker, Eugene Braunwald, Herbert L. Tanenbaum, Andrew G. Morrow

Other Investigators: Russell Holland, Ray Waters

Cooperating Units: Surgery Branch

Man Years (calendar year 1957):
Total: 1
Professional: .50
Other: .50

Patient Days (calendar year 1957):
None

Project Description:

Following the clinical demonstration that indicator dilution curves with left heart injection were useful in the localization of left-to right circulatory shunts, an experimental project was designed to test the validity of this method in animals with proved shunts. Various types of left-to-right shunts were constructed in dogs. These included anomalous pulmonary venous drainage, atrial septal defects, atrial septal defects with mitral insufficiency, ventricular septal defects, aortic insufficiency, aortico-pulmonary window and patent ductus arteriosus. Following construction of the defects, left heart catheterizations and aortic catheterizations were carried out in the dogs and indicator dyes injected at various locations. Dye dilution curves were obtained from the femoral artery by means of a constantly recording densitometer. In normal dogs, injection of the indicator dye into the left atrium, left ventricle or thoracic aorta yielded a dilution curve and a smooth, rapid ascent and descent. In animals with left-to-right shunts, when injections were made from areas proximal to the shunt a fraction of the indicator passed through the pulmonary circulation and in all instances distinctly interrupted the smooth, rapidly descending limb of the primary curve resulting in a secondary peak. The secondary peak was due to that portion of the injected dye passing through the pulmonary circulation. Injections made distal to the shunt, however, yielded normal curves. Thus, by selective injection at various sites,

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PHS-NIH
Individual Project Report
Calendar Year 1957

Project Description (cont.)

the left-to-right shunts could be localized. In those animals with valvular regurgitation, dye injections in the chamber distal to the insufficient valve, resulted in a curve with a normal ascent but a smooth and prolonged descent. It was not always possible, however, to differentiate valvular insufficiency from left-to-right shunts. In each animal in which a left-to-right shunt of known type had been constructed, it could be correctly localized by indicator dilution technique. Thus, on an experimental basis, the validity of the method as clinically applied was proved.

Project has been terminated.

Next Report Included Yes [X] No [ ]

Serial No. NHI-222

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Baker, R. R., Braunwald, E., Tanenbaum, H. L., and Morrow, A. G.: The localization of left-to-right cardiac shunts. An experimental study of indicator dilution curves following left heart and aortic injections. In press. Annals of Surgery.

Form No. OHP-2
Oct. 1957

Serial No. NHI-223 (c)
1. GMFT
2. Cardiodynamics
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A:

Project Title: Pharmacodynamic Studies in the Pulmonary Circulation

Principal Investigators: Samuel M. Fox, 3rd, J. Richard Crout

Other Investigators: Frederick A. Bullock, A. Freeman Bradley, Estelle Cohen, Katherine P. Hirshfield, Rodman L. Turner, William A. Laughlin, Clara V. King

Cooperating Units: Surgery Branch

Man Years (calendar year 1957):
Total: .68
Professional: .41
Other: .27

Patient Days (calendar year 1957): 80

Project Description:

This study was initiated in April 1957 with evaluation of the effects of secondary Amines reported to have a specific pulmonary arterial vasodilatation effect when given to patients with severe precapillary pulmonary hypertension.

One study with Methoxamine ("Vasoxyl") and three with a new compound from the Burroughs-Wellcome Laboratories have been completed.

Systemic vasoconstriction was demonstrated with all compounds in the dosages given and no significant pulmonary vascular change was noted. Suggestive evidence of benefit was found with the single study using Methoxamine, and further investigations are contemplated.

Part B included Yes [ ] No [X]

Form No. ORP-2
Oct. 1957

Serial No. NHI-224

1. GMET
2. Cardiodynamics
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Hemodynamic Studies

Principal Investigators: Samuel M. Fox, 3rd, Herbert L. Tanenbaum, Andrew G. Morrow

Other Investigators: Clara V. King, Estelle Cohen, Fred A. Bullock

Cooperating Units: Surgery Branch

Man Years (calendar year 1957):
Total: .85
Professional: .60
Other: .25

Patient Days (calendar year 1957):
None

Project Description:

a) Dye dilution techniques have been adapted for simultaneously measuring flow and pressure during left heart catheterization and left heart surgery. This makes it possible with reasonable accuracy to quantitate the effective orifice size of the mitral and/or aortic valves. This technique has been used (1) to evaluate the effectiveness of surgical intervention at the time of or after surgery, (2) to determine the role of presenting pressures in the opening of abnormal and fibrous valve leaflets, (3) to study the relationship of hemodynamic factors to such clinical phenomena as the opening snap of mitral stenosis, gallop, third heart sounds, etc. Heretofore, pressure measurements alone were available and none of the above phenomena could be definitively explained by such data in the absence of simultaneous measurements of flow. So far over 20 patients have been so studied. One of the interesting findings has been the observation that when cardiac output is low, as during mitral and aortic stenosis surgery, the effective orifice is smaller than later when output is higher. Evidently the fibrous alteration of the valve leaflet is itself a source of some degree of obstruction to flow.

b) Leaving catheters in place in the left heart and using dye dilution techniques, studies are underway of the effects of pressor agents, cardiac stimulating drugs, and other types of stress on aortic and mitral valvular insufficiency in order further to explore the clinical implications of the studies of Drs. Braunwald and Sarnoff on the effects of ventricular function on experimental valvular insufficiency. It is planned to use the Fry-Wallis flowmeter in these clinical studies. So far ten patients have been studied in this fashion and the results are being analyzed.

Part B included Yes ☐ No ☒

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A:

Project Title: The Pathogenesis of Chronic Diffuse Obstructive Pulmonary Emphysema.

Principal Investigators: Donald L. Fry, Robert E. Hyatt, Donald P. Schilder

Other Investigators: Alexander J. Hallos, Alfred Caspar, Jean Caha

Cooperating Units: None

Man Years (calendar year 1957):
Total: 1.01
Professional: .66
Other: .35

Patient Days (calendar years 1957):
None

Project Description:

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 bronchiolar abnormality that may be either congenital or acquired. Experimentally the objective of these studies have been 1) to establish the nature and magnitude of the stress distribution, 2) to establish the magnitude and distribution necessary to produce disruptive lesions, 3) the nature of the bronchiolar abnormality.

1) The nature and magnitude of the stress distribution was and is being studied in two ways. The dynamic aspect of the problem was studied by measuring the harmonic spectrum of the respiratory flow patterns in human subjects. It was found that the frequency amplitude content of these patterns was such that abnormal stress distribution due to acceleration gradients is extremely unlikely. Abnormal stresses are therefore related to motions not accelerations. This project was begun in September 1956 and ended in March 1957.

To study the stress of motions, descrete stress measurement in animal preparations is being carried out. Small balloons are used to explore the intrathoracic cavity stresses under the parietal pleura as well as along the mediastinum and intralobar surfaces under various rates of ventilation. This project began in September 1957 and is continuing. It is too early to make any significant comment on results.

Form No. ORP-2
Oct. 1957

Serial No. NHI-226
1. GMBT
2. Cardiodynamics
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Clinical and Experimental Electrocardiography

Principal Investigators: Robert P. Grant, Thomas N. Lynn, Samuel M. Fox, III

Other Investigators: Clara V. King, Margaret Austin

Cooperating Units:

Man Years (calendar year 1957):
Total: .70
Professional: .45
Other: .25

Patient Days (calendar year 1957):
None

Project Description:

a) Thirty cases of the pre excitation syndrome with tracings during normal and abnormal conduction have been collected from various hospitals, and twenty more from the literature, representing the first controlled study of the QRS electrical changes which take place in this syndrome. The analysis of this material has lead to more critical and accurate definition of the abnormality of ventricular activation in the Wolff-Parkinson-White syndrome. Proof has been found of a relatively unusual type of ventricular conduction defect in certain types of WPW. This material is now being written up for publication.

b) A continuing study of the ECG manifestations of congenital heart disease has been extended to the use of intra-cavity measurements with specially prepared electrodes and special techniques for relating the findings to the body surface ECG. The goals of this line of work are: (1) to identify the source of the electrical potential responsible for certain wave forms seen in right ventricular hypertrophy; (2) the elucidation of the mechanism of the unique and rather diagnostic ECG seen in the persistent ostium primum type of atrial septal defect; (3) a cataloguing of ECG's in congenital heart disease employing the technique of dissecting of the components of the spatial QRS loop - a more accurate technique than has been used heretofore for this purpose, (4) the study of

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Individual Project Report
Calendar Year 1957

Project Description (cont.):

the effects on electrical forces of surgical correction of congenital cardiac defects, leading to techniques for differentiating the electrical effects of hypertrophy from those of dilatation. These aspects of the ECG program are based upon the wealth of congenital cardiac material on the surgical service, with over 60 autopsied cases, plus the material at Children's Hospital made available to us by Dr. Robert Parrott and his staff. This program was initiated July 1957, and is in its earliest stages.

Part B included Yes [X] No [ ]

Serial No. NHI-226

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Grant, R. P., Clinical Electrocardiography, A Spatial Vector Approach, 1st Edition, New York City, McGraw-Hill Book Company, November 1957.

Form No. OBP-2
Oct. 1957

PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
Surgery Branch

Estimated Obligations for FY 1958

Total:	$976,748
Direct:	$230,169
Reimbursements:	746,579

Form No. ORP-2
Oct. 1957

Serial No. NHI-227 (c)
1. Surgery Branch
2. None
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Left Heart Catheterization by the Transbronchial Route.

Principal Investigator: Andrew G. Morrow, M. D.

Other Investigators: Eugene Braunwald, M. D.

Technical: Estelle R. Cohen, Fred Bullock & William Laughlin

Cooperating Units: None.

Man Years (calendar year 1957): Patient Days (calendar year 1957): 1760
Total: 2.5
Professional: 2
Other: 0.5

Project Description: Numerous previous reports have dealt with the technique and applications of left heart catheterization in the assessment of patients with various cardiovascular defects. To date, more than 600 transbronchial left heart catheterizations have been performed. There have been no deaths or serious sequelae from the procedure. The two general areas of investigation have been those concerned with primary physiologic studies in which the information obtained was not necessarily related to the patient's treatment. These have included the determination of the sequence of ventricular contraction in bundle branch block, the study of pressure-volume relationships in the left atrium and left ventricle, the effect of increased peripheral resistance on the contour of the left atrial pressure pulse, particularly in patients with mitral valve disease, and the application of indicator dilution techniques in the localization of left-to-right shunts with left heart injection.

The second area of clinical interest has been in the study of patients with valvular heart disease. It has been possible to establish criteria for the evaluation of the left atrial pressure pulse contour in differentiating mitral stenosis from mitral insufficiency. Three formulae applied to left atrial pressure curves have been applied and their validity proved in more than 50 patients with valve lesions of known type. The characterization of stenotic valvular lesions has been enhanced by the combination of cardiac output determination with pressure measurements at the time of left heart catheterization. Cardiac output is performed with left ventricular injection of indicator dilution materials as curves are recorded from a densitometer connected to the femoral artery. When both the pressure gradient and the cardiac output are known, fairly precise estimates of the area of the stenotic orifice can be determined and the

necessity for treatment more adequately determined before operation. The procedure has obvious value in the assessment of the results of operation for mitral stenosis and aortic stenosis and all patients subjected to these operations have been studied six to twelve months postoperatively.

Current researches are directed at determining the effects of the procedure itself on the patient's metabolic and physiologic state. Determinations of oxygen consumption and cardiac output are being made at various intervals during the course of transbronchial left heart catheterization. Preliminary data in 8 patients indicates that a steady basal state can be achieved within 20 minutes after bronchoscopy and while the catheter remains in the left atrium and left ventricle. Determination of cardiac output at various stages in the procedure indicate that bronchoscopy itself acts as a minimal physiologic stress in most patients. The cardiac index, for example, was elevated only 330 cc./min./M^2 while the bronchoscope was in place. It has been effectively proved that the transbronchial method of left heart catheterization is preferable to other techniques since it combines the advantages of safety with the ability to make extended observations with the patient in a known basal physiologic state.

Part B included Yes [X] No [ ]

Serial No. NHI-227 (c)

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards and Publications

Morrow, A. G., Braunwald, E. & Tanenbaum, H. L.: Transbronchial left heart catheterization. A modified technique and its physiologic evaluation. In press. A.C.S.

Morrow, A. G., Braunwald, E., Haller, J. A., Jr. & Sharp, E. H.: Left heart catheterization by the transbronchial route. Technique and applications in physiologic and diagnostic investigations. Circulation 16:1033-1039, Dec. 1957.

Ross, J., Braunwald, E. & Morrow, A. G.: Clinical and hemodynamic observations in pure mitral insufficiency. In press. Am. J. Cardiology.

Braunwald, E., Welch, G. H. & Morrow, A. G.: The effects of acutely increased systemic resistance on the left atrial pressure pulse. A method for the clinical detection of mitral insufficiency. In press. J. of Clin. Investigations.

Braunwald, E. & Morrow, A. G.: Sequence of ventricular contraction in human bundle branch block. A study based on simultaneous catheterization in both ventricles. Am. J. Med. 23:205-211, August 1957.

Morrow, A. G., Braunwald, E., Haller, J. A., Jr. & Sharp, E. H.: Left atrial pressure pulse in mitral valve disease. A correlation of pressures obtained by transbronchial puncture with the valvular lesion. Circulation 16:399-405, Sept. 1957.

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Oct. 1957

Serial No. NHI-228 (c)
1. Surgery Branch
2. None
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Use of Radioactive Trifluoroiodomethane and Kr^{85} for the Diagnosis of Cardiac Shunts.

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

Other Investigators: Andrew G. Morrow, M. D.

Technical: Fred Bullock & Estelle R. Cohen

Cooperating Units: None.

Man Years (calendar year 1957): Patient Days (calendar year 1957): 900
Total: 3
Professional: 2
Other: 1

Project Description: After it has been demonstrated that an inert gas, nitrous oxide, could be used in the localization of left-to-right shunts, it was thought desirable to substitute for nitrous oxide a radioactive gas. The advantages of a radioactive gas appeared to be that the samples can be analyzed immediately and the results of the test obtained while the catheter is still in the heart. An experimental study was designed in which ten normal dogs and ten dogs with atrial and ventricular septal defects were studied. As the dogs inhaled radioactive trifluoroiodomethane samples were drawn from an appropriate right heart chamber and the femoral artery. In the absence of a left-to-right shunt, the radioactivity of the right heart samples was always less than 10% of the arterial sample. In dogs with shunts the radioactivity of the right heart samples always exceeded 10%.

Investigations continue with this and other gases concerning the optimum period of sampling, the significant levels at which a left-to-right shunt may be diagnosed.

Part B included Yes [X] No [ ]

Serial No. NHI-223 (c)

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards and Publications,

Sanders, R. J.: The use of radioactive gas (Kr^{85}) in the diagnosis of cardiac shunts. In press. Proc. Soc. Exper. Bio. & Med.

Form No. CRP-2
Oct. 1957

Serial No. NHI-229 (c)
1. Surgery Branch
2. None
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Use of Indicator-Dilution Curves from the Left Heart and Aorta in the Localization of Left-to-Right Shunts in Patients.

Principal Investigator: Andrew G. Morrow, M. D.

Other Investigators: Eugene Braunwald, M. D. & Herbert L. Tanenbaum, M. D.

Technical: Estelle R. Cohen & Fred Bullock

Cooperating Units: None.

Man Years (calendar year 1957): Patient Days (calendar year 1957): 1300
Total: 3
Professional: 2
Other: 1

Project Description: The precise localization of the origin of the left-to-right shunt may be difficult by determination of the oxygen differences from the cardiac chambers even when an inert gas, such as nitrous oxide, is used. These techniques are not applicable in the detection, for example, of valvular insufficiency in association with the left-to-right shunts. The techniques of left heart catheterization and aortic catheterization have made possible the injection of indicator dye into the left atrium and left ventricle and various sites in the aorta. Indicator dilution curves have been obtained from these sites and their value assessed in the localization of left-to-right shunts.

More than 100 patients have now been studied. In the absence of a left-to-right shunt, or valvular regurgitation, a left heart aortic dye injection results in a primary curve with a sharp, rapid ascent and descent. When injection is made proximal to the site of the shunt, a portion of the dye passes through the pulmonary circulation and interrupts the descending limb of the dye curve with the secondary peak. By selective injections at various locations the origin of the left-to-right shunt can be detected. Similarly, valvular insufficiency results in a prolonged, but smooth, descending limb of the dye curve and by selective injection, valvular insufficiency may also be localized. By combining dye injections with the technique of cardiac catheterization and the use of the nitrous oxide test, we are presented with numerous method for localizing the shunt.

A problem has presented itself in the detection of small shunts and the differentiation of them from valvular insufficiency. The curves are presently being recalibrated on semi-logarithmic paper and the slopes of the ascending and descending limbs being compared. It is believed that this will result in more precise methods of analysis of the results in curves. The investigations are being continued in an increasing number of patients.

Part B included Yes [X] No [ ]

Serial No. NHI-229 (c)

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards and Publications.

Grant, R. P., Sanders, R. J., Morrow, A. G. & Braunwald, E.: Symposium on diagnostic shunts in the study of left-to-right shunts. Circulation 16:791-802, Nov. 1957.

Braunwald, E., Tanenbaum, H. L. & Morrow, A. G.: Localization of left-to-right cardiac shunts by dye dilution curves following injections into the left heart and aorta. In press. Am. J. Med.

Baker, R. R., Braunwald, E., Tanenbaum, H. L. & Morrow, A. G.: The localization of left-to-right cardiac shunts. An experimental study of indicator dilution curves following left heart and aortic injections. In press. Annals of Surgery.

Wood, J. C., Conrad, M. E., Jr. & Morrow, A. G.: Partial anomalous venous connection. A case report illustrating diagnostic techniques. Am. Heart J. 54:422-428, Sept. 1957.

Braunwald, E., Tanenbaum, H. L. & Morrow, A. G.: Dye-dilution curves from left heart and aorta for localization of left-to-right shunts and detection of valvular insufficiency. Proc. of the Soc. for Exper. Bio. & Med. 94:510-512, 1957.

Form No. ORP-2
Oct. 1957

Serial No. NHI-230 (c)
1. Surgery Branch
2. None
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Experimental Evaluation and Clinical Application of the Melrose Artificial Heart and Lung Machine.

Principal Investigators: Edward H. Sharp, M. D.

Other Investigators: John Ross, Jr., M. D.
Clarence Weldon, M. D.
Joseph W. Gilbert, Jr., M. D.
Andrew G. Morrow, M. D.

Technical: William Laughlin
Freeman Bradley
Fred Bullock
Robert L. White
Leander Brown
Russell Holland

Cooperating Units: None.

Man Years (calendar year 1957): Patient Days (calendar year 1957): 900
Total: 4
Professional: 3
Other: 1

Project Description: One year ago a Melrose artificial heart and lung machine was obtained from England. This is a device which oxygenates blood by its passage through a cylinder containing numerous rotating plates. Preliminary experimentation with the machine in animals showed very low survival rates. Following the study of these animals, numerous modifications in the instrument were made. These included: 1. the institution of gravity drainage of venous blood into the machine rather than pumping of venous blood, 2. the addition of an efficient air trap, 3. standardization of tubing used, 4. precise calibration of the oxygenation capacity of the artificial lung and, 5. the substitution of roller-type DeBakey pumps for the Melrose pumps originally supplied with the machine. With these modifications, survival was obtained in approximately 80% of animals perfused for 30 minutes. Since February 1956, 20 patients have been perfused with the aid of the machine in its present form. Approximately one-third of these are survivors. Two obvious defects in the machine have become apparent with this degree of clinical use. Of the patients operated upon, five have had massive bleeding in the postoperative period. The explanation for this has not been clear, but it is probably associated with inadequate oxygenation of tissues resulting in capillary damage. A detailed hematologic investigation is now underway concerning this

coagulation defect. The other defect which is related to the first, is that the machine is probably not capable of oxygenating more than 2500 to 3000 cc. of blood per minute. Thus it is not adequate for use in adults of large body size. The problem of air embolism which was early a difficult one has largely been solved and it remains to be seen whether or not the coagulation defect noted in the five patients is due to the fundamental design of the instrument or whether it can be attributed to mismanagement of heparin and protamine in the care of patients. Bleeding has not been a problem in the experimental animal. At the present stage it has been decided to discontinue clinical use of the artificial heart and lung machine except in exceptional instances.

Current researches are being directed at the establishment of an internal circuit in the machine which will provide for constant circulation of blood into and out of the oxygenator but arterial blood supply to the patient will be from an arterial blood reservoir rather than from the oxygenator itself. This appears to have the dual advantage of providing larger oxygenation capacity and also will provide additional safeguards against air embolism since the arterial blood will be pumped from a reservoir which is still rather than from a rotating pool such as in the machine in its present form.

Current researches in the field of pump oxygenation are not being principally directed at the Melrose machine. It has become obvious from research in various areas that a membrane-type oxygenator in which blood and gas do not come into direct contact with each other is probably preferable. Several membranes, including polyethylene, Teflon and Silastic have proved useful in the experimental laboratory. The problem now remains to package these membranes in an economic fashion to provide sufficient surface areas with blood. It is to be hoped that within the next several months that an efficient membrane-type oxygenator will be available for clinical use.

Part B included Yes/ / No /X/

Form No. ORP-2
Oct. 1957

Serial No. NHI-231
1. Surgery Branch
2. None
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Experimental Proof of the Value of Indicator Dilution Curves in the Localization of Left-to-Right Circulatory Shunts.

Principal Investigator: R. Robinson Baker, M. D.

Other Investigators: Eugene Braunwald, M. D.
Herbert L. Tanenbaum, M. D.
Andrew G. Morrow, M. D.

Technical: Russell Holland & Ray Waters

Cooperating Units: None.

Man Years (calendar year 1957): Patient Days (calendar year 1957): None.
Total: 1
Professional: 0.5
Other: 0.5

Project Description: Following the clinical demonstration that indicator dilution curves with left heart injection were useful in the localization of left-to-right circulatory shunts, an experimental project was designed to test the validity of this method in animals with proved shunts. Various types of left-to-right shunts were constructed in dogs. These included anomalous pulmonary venous drainage, atrial septal defects, atrial septal defects with mitral insufficiency, ventricular septal defects, aortic insufficiency, aortico-pulmonary window and patent ductus arteriosus. Following construction of the defects, left heart catheterizations and aortic catheterizations were carried out in the dogs and indicator dyes injected at various locations. Dye dilution curves were obtained from the femoral artery by means of a constantly recording densitometer. In normal dogs, injection of the indicator dye into the left atrium, left ventricle or thoracic aorta yielded a dilution curve and a smooth, rapid ascent and descent. In animals with left-to-right shunts, when injections were made from areas proximal to the shunt a fraction of the indicator passed through the pulmonary circulation and in all instances distinctly interrupted the smooth, rapidly descending limb of the primary curve resulting in a secondary peak. The secondary peak was due to that portion of the injected dye passing through the pulmonary circulation. Injections made distal to the shunt, however, yielded normal curves. Thus, by selective injection at various sites, the left-to-right shunts could be localized. In those animals with valvular regurgitation, dye injections in the chamber distal to the insufficient valve, resulted in a curve with a normal ascent but a smooth and prolonged descent. It was not always possible, however, to

differentiate valvular insufficiency from left-to-right shunts. In each animal in which a left-to-right shunt of known type had been constructed, it could be correctly localized by indicator dilution technique. Thus, on an experimental basis, the validity of the method as clinically applied was proved.

Project has been terminated.

Part B included Yes [X] No [ ]

Serial No. NHI-231

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards and Publications.

Baker, R. R., Braunwald, E., Tanenbaum, H. L. & Morrow, A. G.: The localization of left-to-right cardiac shunts. An experimental study of indicator dilution curves following left heart and aortic injections. In press. Annals of Surgery.

Form No. ORF-2
October 1957

Serial No. NHI-232 (c)
1. Surgery Branch
2. None
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Nitrous Oxide Test. An Improved Method for the Diagnosis of Left-to-Right Circulatory Shunts.

Principal Investigator: Andrew G. Morrow, M. D.

Other Investigators: Richard J. Sanders, M. D. & Eugene Braunwald, M. D.

Technical: Estelle R. Cohen & Fred Bullock

Cooperating Units: None.

Man Years (calendar year 1957): Patient Days (calendar year 1957): 1[illegible]
Total: 2
Professional: 1.5
Other: 0.5

Project Description: Previous reports have outlined the usefulness of an inert gas, nitrous oxide, in the localization of left-to-right circulatory shunts. The advantages of this method over the determination of oxygen differences has been proved. The test is applied by having a patient inhale nitrous oxide as samples are drawn simultaneously from one of the cardiac chambers or the pulmonary artery and the femoral artery. In patients without shunts the ratio of right heart samples with arterial samples has always been less than 20%. In patients with left-to-right shunts, a ratio of right atrial to arterial levels are 30% or more and a pulmonary artery or right ventricular to arterial level are 20% or more have been established as diagnostic of left-to-right shunts. The test has been carried out in more than 200 patients and in 148 patients, both the nitrous oxide test and oxygen differences were compared. In these patients the nitrous oxide test made only 3 diagnostic errors while 21 diagnostic errors were made on the basis of oxygen differences.

The original technique of administration of the gas required the inhalation of 15% nitrous oxide and samples were drawn over a period of one minute. In the past year the changes in this concentration and sampling periods of nitrous oxide have been carried out. The present technique involves the inhalation of 50% nitrous oxide and the blood samples being drawn between the 20th and 30th seconds of inhalation.

Further refinement of the mode of administration of the gas will be necessary and it is likely that new diagnostic studies will be set up.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards and Publications.

Morrow, A. G., Sanders, R. J. & Braunwald, E.: The Nitrous Oxide Test. An Improved Method for the Detection of Left-to-Right Shunts. In press. Circulation.

Form No. CRP-2
Oct. 1957

Serial No. NHI-233
1. Surgery Branch
2. None
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Reversal of the Cardiac Effects of Several Sympathomimetic Amines by Digitalis Glycosides During Hypothermia.

Principal Investigator: Theodore Cooper, M. D., Ph.D.

Other Investigators: Marion deV. Cotten, Ph.D.

Technical: Phyllis E. Stepp

Cooperating Units:

Man Years (calendar year 1957): Patient Days (calendar year 1957): None.
Total: 1
Professional: 1
Other:

Project Description: Previous experiments have shown that the effects of sympathomimetic amines on the blood pressure and cardiac contractile force are relatively unchanged as the body temperature of dogs is lowered from 37° C. to 20° C. The effects of cardiac glycosides on the contractile force are not particularly changed at body temperatures in the range of 30° C. but are significantly reduced at body temperatures of 25° C. and lower. Incidental to these experiments, it was noted that the administration of epinephrine following ouabain at body temperatures of between 28-30° C. resulted in a decrease in contractile force rather than the usual stimulant action. Additional experiments were conducted to confirm and extend this observation. Analysis of data from this group of experiments has proceeded. The data is consistent, i.e., the cardiac effects of the sympathomimetic amines are reversed by the digitalis glycosides at body temperatures of 30° C. (R). Preliminary attempts at in vitro experimentation on the papillary muscle of the cat have not been satisfactory from the technical standpoint.

Completion of the analysis of the data are in order. Further attempts at instrumenting a satisfactory papillary muscle preparation will be made.

Part B included Yes [ ] No [X]

Form No. ORP-2
Oct. 1957

Serial No. NHI-234 (c)
1. Surgery Branch
2. None
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies in the Detection and Estimation of the Volume of Aortic Regurgitant Flow in Man.

Principal Investigator: Eugene Braunwald, M. D.

Other Investigators: Andrew G. Morrow, M. D.

Technical: Estelle R. Cohen & Fred Bullock

Cooperating Units: None.

Man Years (calendar year 1957): Patient Days (calendar year 1957): 700
Total: 2
Professional: 1
Other: 1

Project Description: Estimates have been made for many years of the actual volume of blood being regurgitated across the aortic valve in the presence of aortic insufficiency. Experiments in the laboratory of cardiovascular physiology have indicated that large regurgitant volumes may be compatible with low ventricular end-diastolic pressure and consequent low left atrial pressure. The following method has been devised to estimate aortic regurgitant flow in man. An oximeter is placed on the patient's right ear. A cardiac catheter is then passed percutaneously from the femoral artery into the aortic arch and dye injections are made at various levels in the aortic arch and in the descending aorta. The catheter is gradually withdrawn and the end-point in the aorta from which dye regurgitates into the ascending aorta and appears at the right ear is determined. The position of the catheter is then recorded by x-ray. From anatomic studies in the literature it has been possible to calculate the volumes of the various aortic segments and by the application of these volumes and the patient's mean diastolic aortic pressure an estimate of the regurgitant volume can be arrived at. In addition to estimating the volume of aortic regurgitants in patients with aortic insufficiency the method has proved useful in differentiating aortic insufficiency of minimal degree from pulmonic insufficiency. The technique has also been useful in the preoperative assessment of the degree of aortic insufficiency produced at the time of aortic commissurotomy.

The method has been established as a useful clinical tool but further refinements in the method of calculating the volume of regurgitant flow are being investigated.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards and Publications.

Morrow, A. G., Braunwald, E. & Sharp, E. H.: The clinical features and surgical treatment of congenital aortic stenosis. In press. Progress in Cardiovascular Diseases.

Braunwald, E. & Morrow, A. G.: A method for the detection and estimation of aortic regurgitant flow in man. In press. Circulation.

Card No. [illegible]
1. Surgery Branch
2. None
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Simultaneous Determinations of Pressure Gradient and Cardiac Output During Operation as a Means of Immediate Assessment of the Efficacy of Operations for Stenotic Valvular Disease.

Principal Investigator: Andrew G. Morrow, M. D.

Other Investigators: Herbert L. Tanenbaum, M. D. & Eugene Braunwald, M.

Technical: Estelle R. Cohen, William Laughlin & Fred Bullo

Cooperating Units: None.

Man Years (calendar year 1957): Patient Days (calendar year 1957):
Total: 2.5
Professional: 2
Other: 0.5

Project Description: In stenotic lesions of the mitral and aortic valves flow across the valve is turbulent. Under these circumstances, the interpretation of a pressure gradient is impossible if the flow across the valve is not known. Accordingly, a technique has been designed for the sequential determination of valve gradients and cardiac output at the time of operation for stenotic valvular lesions. After the heart has been exposed at the operating table, pressures are measured simultaneously in the various cardiac chambers. In mitral stenosis, pressures are measured in the left atrium and left ventricle; in aortic stenosis, the pressures are measured in the left ventricle and aorta; in pulmonic stenosis, pressures are measured in the right ventricle and pulmonary artery and in tricuspid stenosis, pressures are measured in the right atrium and right ventricle. Immediately following measurement of the pressure gradient cardiac output is determined by the indicator dilution technique with left atrial or pulmonary venous injection. The procedure is repeated following the commissurotomy. A gross estimate of the efficacy of the operation can be obtained by visual monitoring of this oscilloscopic trace of the pressure gradients and of the indicator dilution curve. A fixed amount of dye is used and thus, increased cardiac output is reflected by a smaller inscribed curve. Thus an immediate estimate of the increase in flow and diminution of pressure gradient can be obtained. Later calculation of the estimated valve area gives the most precise definition of the efficacy of the operation. The procedure has been of particular importance in operations carried out under general hypothermia since cardiac output is significantly reduced under these circumstances. The procedure has been carried out in nearly 40 patients undergoing operations for stenotic valvular lesions.

The procedure is being continued and refinements in the technique being made.

Part B included Yes [X] No [ ]

Serial No. NHI-235 (c)

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards and Publications.

Tanenbaum, H. L., Braunwald, E. & Morrow, A. G.: Determination of cardiac output and valve gradients at operation. A technique for the immediate assessment of the results of operations for stenotic valvular disease. In press. New England Journal of Medicine.

Form No. CRP-2
Oct. 1957

Serial No. NHI-236
1. Surgery Branch
2. None
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Transatrial Catheterization of the Left Heart.

Principal Investigator: John Ross, Jr., M. D.

Other Investigators: Technical: William Laughlin

Cooperating Units: None

Man Years (calendar year 1957): Patient Days (calendar year 1957): None
Total: 0.5
Professional: 0.5
Other:

Project Description: Studies have been initiated to devise an instrument for and to study the technique of catheterization of the left atrium by needle puncture through the interatrial septum. A cardiac catheter with a long retractable metal needle has been constructed. By passing the catheter fluoroscopically through the femoral vein in 30 dogs and by direct vision via the femoral vein in 8 dogs undergoing thoracotomy, the left atrium has been successfully entered about 90% of the attempts.

At present an attempt to construct a more satisfactory catheter is in progress. Additional dogs will be catheterized. Postmortem examinations for evidence of damage to the interatrial septum are being carried out.

Part B included Yes [ ] No [X]

Form No. ORP-2
Oct. 1957

Serial No. NHI-237
1. Surgery Branch
2. None
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Bronchial Arterial Flow in an Isolated Lobe of the Lung.

Principal Investigator: John Ross, Jr., M. D.

Other Investigators: Technical: William Laughlin & Ray Waters

Cooperating Units: None.

Man Year (calendar year 1957): Patient Days (calendar year 1957): None.
Total: 0.25
Professional: 0.25
Other:

Project Description: The purpose of the investigation is to study the hemodynamics and physiologic significance of bronchial arterial flow into a lobe of the lung to which pulmonary arterial flow has been stopped. Sixteen dogs have been studied to date. The pulmonary artery and pulmonary vein to one lobe of the lung were isolated, a control biopsy of the lobe was taken and pulmonary artery and pulmonary venous pressures were measured. The pulmonary vein and the pulmonary artery were then gradually occluded as pressures in these vessels were measured. It was found that pressures in both vessels gradually rose over the course of 45 minutes when both vessels were completely occluded. This was associated with hemorrhagic and edematous changes in the isolated lobe. It was found also that release of either the pulmonary artery or the pulmonary vein prevented these pressure rises and subsequent histologic changes in the lung. The relationship of these studies to the studies relating to cardiac bypass and pulmonary pathology is obvious.

This project has been discontinued and has been continued in the form outlined under cardiac arrest.

Part B included Yes [ ] No [X]

Form No. ORP-2
Oct. 1957

Serial No. NIH-238
1. Surgery Branch
2. None
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

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, Jr., M. D.
Edward H. Sharp, M. D.
Clarence Weldon, M. D.
Andrew G. Morrow, M. D.

Technical: Russell Holland
Ray Waters
Robert White
William Laughlin

Cooperating Units: None.

Man Years (calendar year 1957): Total: 2
Professional: 1.5
Other: 0.5

Patient Days (calendar year 1957): 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, dogs have been 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 have been performed before and after perfusion. Investigation to date has shown that control dogs without cardiac arrest or cardiotomy showed no pressure elevations in the cardiac chamber. However, in hearts subjected to cardiac arrest and without cardiotomy significant rises in left atrial and right ventricular pressures were noted. In addition, these animals showed complications during the recovery period such as ventricular fibrillation and cardiac failure. In the lungs 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.

Investigations are being continued with attempts being made to determine which portion of left-sided cardiac return during occlusion is due to bronchial arterial circulation and which to other sources.

Part B included Yes [ ] [X]

Form No. ORP-2
Oct. 1957

Serial No. NHI-239
1. Surgery Branch
2. None
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Intrapulmonary Transplantation of Autogenous Thyroid Tissue per Cournand catheter.

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

Other Investigators: Joseph W. Gilbert, Jr., M. D.

Cooperating Units: None.

Man Years (calendar year 1957): Patient Days (calendar year 1957): None.
Total: 0.25
Professional: 0.25
Other:

Project Description: Studies have been initiated to determine whether homogenized glandular tissue may be successfully transplanted per Cournand catheter to the pulmonary vascular bed. Hemithyroidectomy has been carried out in 30 dogs, with injection of the ground up pieces of gland into a lobar artery, through a cardiac catheter. Twenty-two of these dogs have been sacrificed, and in only 2 or 3 is there evidence of thyroid tissue in the lungs.

Eight dogs remain, and in these, total thyroidectomies have been done to stimulate any thyroid gland growing in the lung. It is planned to give these dogs I^{131} in 3 or 4 months, to localize any thyroid that might be growing there, and then section these pieces of lung for microscopic study.

Part B included Yes [ ] No [X]

Form No. ORP-2
Oct. 1957

Serial No. NHI-240
1. Surgery Branch
2. None
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Experimental Pulmonary Emboli and Their Relationship to Serotonin.

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

Other Investigators: Joseph W. Gilbert, Jr., M. D.

Cooperating Units: None.

Man Years (calendar year 1957): Patient Days (calendar year 1957): None
Total: 0.25
Professional: 0.25
Other:

Project Description: The purpose of the investigation is to study the effects of sublethal pulmonary emboli in dogs, and to compare these with the effects of serotonin, to determine whether or not emboli act by releasing serotonin. A method for producing physiological emboli in the jugular veins has been devised, using homologous serum from another dog. The pressure changes produced by the release of these emboli have been recorded in the left atrium, femoral artery, and pulmonary artery. As a basis for comparison, similar pressures have been recorded when serotonin was injected and when mechanical occlusion of one of the pulmonary or lobar arteries was produced with tapes. The changes suggest that in dogs, the pressure changes from pulmonary embolization closely simulate the changes of mechanical occlusion, rather than those of serotonin injection.

To further confirm the above impression, measurements of serum serotonin level after release of emboli are being carried out. If the above impressions are correct, the serum should show no increase in serotonin with the release of emboli. We also plan to study serotonin antagonists.

Part B included Yes [ ] No [X]

Form No. CRP-2
Oct. 1957

Serial No. NHI-241
1. Surgery Branch
2. None
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the Excitability of Ventricular Heart Muscle During Hypothermia with and without Procaine Blockade of the Caval-Atrial Junction.

Principal Investigator: Theodore Cooper, M. D.

Other Investigators: None.

Cooperating Units: None.

Man Years (calendar year 1957): Patient Days (calendar year 1957): Non
Total: 0.5
Professional: 0.5
Other:

Project Description: Previous reports from this laboratory have shown the beneficial effect on the prevention of ventricular fibrillation during hypothermia and ventriculotomy of caval-atrial blockade with procaine. This study is an effort to determine whether this effect is due to an alteration in the electrical excitability of the ventricular muscle and to determine the source of this change if present. Design of a satisfactory electrical system for quantitative evaluation of thresholds for premature ventricular contractions and ventricular fibrillation has been effected. The instrumentation is currently being assembled.

The study of the excitability of heart muscle during normal and hypothermia, with and without procaine blockade of the caval-atrial junction will be performed. The effects of other surgical manipulations and medications will also be evaluated.

Part B included Yes [ ] No [X]

Form No. ORP-2
Oct. 1957

Serial No. NHI-242 (c)
1. Surgery Branch
2. None
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Pulsus Alternans in Aortic Stenosis

Principal Investigator: Theodore Cooper, M. D.

Other Investigators: Eugene Braunwald, M. D. & Andrew G. Morrow, M. D.

Cooperating Units: None.

Man Years (calendar year 1957): Patient Days (calendar year 1957): 200
Total: 1
Professional: 1
Other:

Project Description: The phenomenon of pulsus alternans has been noted in many conditions in which the myocardium has been subjected to an increased burden. The mechanism of this phenomenon is poorly understood. During the course of left heart catheterization analysis in acquired valvular heart disease the phenomenon was noted in several records obtained from patients with aortic stenosis. Correlation of the presence and magnitude of this phenomenon with other hemodynamic data and the clinical status of the patient with aortic stenosis may provide some insight into its significance and possibly its mechanism. Program of analysis has been devised. Study of the clinical and catheterization records has been started. Superficial review indicated that the phenomenon is present in those cases with severe disease. No correlation with the size of the aortic valve gradient is yet apparent.

A more complete analysis of related factors is underway.

Part B included Yes [ ] No [X]

Form No. ORP-2
Oct. 1957

Serial No. NHI-243
1. Surgery Branch
2. None
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Reactivity of Cardiac Muscle to Adrenergic Agents After Bilateral Thoracic Sympathectomy.

Principal Investigator: Theodore Cooper, M. D.

Other Investigators: None.

Cooperating Units: None.

Man Years (calendar year 1957): Patient Days (calendar year 1957)
Total: 0.25
Professional: 0.25
Other:

Project Description: Previous attempts to demonstrate alterations in the sensitivity of the "sympathectomized" heart to adrenergic drugs have utilized heart rate and blood pressure responses as a basis for analysis. The strain gauge arch has provided a means whereby the inotropic response of cardiac muscle to l-epinephrine and l-norepinephrine can be evaluated directly. The responses of the intact heart are well documented. The study of additional sympathectomized dogs has been accomplished. No distinct difference in threshold level responses to intravenous l-epinephrine and l-norepinephrine can be distinguished from the normal controls or from those results already recorded in the literature. It appears that the heart is dilated or enlarged at the time of testing as compared to the time of surgery. Note that the pericardium was not violated in these animals. Measurement of intracardiac pressures in a few animals of this group suggests a distinct alteration in pressures.

Analysis of dose responses to the adrenergic agents will be completed and shall be compared to the dose responses of the normal animal.

Part B Included Yes [ ] No [X]

Form No. ORP-2
Oct. 1957

Serial No. NHI-244 (c)
1. Surgery Branch
2. None
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: The Study of Cardiovascular Hemodynamics Before, During and After Intracardiac Surgery, with Special Reference to the Physiologic Effects Following Closure of Atrial and Ventricular Septal Defects.

Principal Investigator: Theodore Cooper, M. D.

Other Investigators: Andrew G. Morrow, M. D. & Eugene Braunwald, M. D.

Cooperating Units: None.

Man Years (calendar year 1957): Total: 0.5
Professional: 0.25
Other: 0.25

Patient Days (calendar Year 1957): 10

Project Description: The nature of the immediate adjustments with which the heart must cope following corrective heart surgery has been poorly documented. Broad statements concerning procedures in management of the postoperative patient and concerning poor results in some cases have been drawn from isolated pressure readings at the operating table, and from pre-operative catheterization data. The current study was undertaken to determine the nature of these cardiovascular adjustments for several hours or days following corrective surgery. It also demonstrates a means whereby postoperative management in this critical period may be guided. The following method has proven workable in several patients. Prior to surgery a catheter is introduced into the right ventricle. A radial artery catheter is inserted in the anesthesia room and EKG electrodes are placed in the usual areas. Immediately following thoracotomy, a left atrial catheter is inserted by way of a pulmonary vein radical. Consequently, EKG, RV pressure, RV (PA or RA) pressure and arterial pressure are measured before, during and after the surgical intervention. This process continues in the recovery room for as long as is necessary for management or until a stable state has been achieved. This is usually in excess of 20 hours.

This method will be applied to additional cases. Analysis may then provide a definite pattern of cardiovascular adjustment which may be anticipated in various heart lesions.

Part B included Yes / / No /X/

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PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
Laboratory of Clinical Biochemistry

Estimated Obligations for FY 1958

Total:	$118,429
Direct:	$85,695
Reimbursements:	32,734

Form No. ORP-2
Oct. 1957

Serial No. NHI-245
1. Laboratory of Clinical Biochemistry
2. None
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Hydroxyproline

Principal Investigator: Dr. Chozo Mitoma

Other Investigators: Dr. Sidney Udenfriend

Cooperating Units:
1. Drs. A. Sjoerdsma and John Davidson, Clinic of General Medicine and Experimental Therapeutics, NHI
2. Drs. B. Witkop and Arthur A. Patchett, Laboratory of Chemistry, NIAMD-59.

Man Years (calendar year 1957):
Total: 4
Professional: 2
Other: 2

Patient Days (calendar year 1957):
None

Project Description:

Contrary to the widely accepted dogma that free hydroxyproline (OPr) is not incorporated into collagen, OPr-C^{14} was shown to be utilized by rapidly developing chick embryos and by artificially induced guinea pig fibrous tissues.

The conversion of proline to OPr is very slow and appears to be weakly catalyzed by kidney homogenates.

Preliminary studies indicate that ascorbic acid is not involved in the hydroxylation of proline, since blood and urinary levels of OPr in scorbutic guinea pigs are not lower than in normals.

A survey of compounds for their possible effects on OPr metabolism was conducted using fertilized eggs. Ketoproline and thiomethylproline raised the free OPr level in chick embryos 2-3 fold while acetyl salicylate and hydrocortisone lowered OPr. In vitro studies indicate that ketoproline partially protects the destruction of OPr but has no effect on proline disappearance.

Analyses of urines from more than 70 patients and controls indicate that patients with Marfan's syndrome (6 out of 9) excrete 2 to 3 times more OPr per day than others. Other conditions in which OPr excretion may be elevated are carcinoids, myeloma and argyria.

PHS-NIH
Individual Project Report
Calendar Year 1957

When 4 gm. of OPr is given orally to a patient, normally little of this appears in the urine. However, patients with Marfan's and Erlos Danlos syndromes tend to spill part of the OPr into the urine.

It was found that the amount of OPr excreted in the urine under normal conditions is not influenced by the oral administration of 4 gm. of OPr or by giving other amino acids, along with the OPr. However, simultaneous administration of glutamic acid casein or gelatin with OPr also results in high excretion of OPr.

In vivo experiments designed to establish the chemical role of ascorbic acid in connective tissue metabolism will be conducted.

If possible, the enzyme system involved in the formation of OPr will be studied in detail.

Effects of hormones, drugs and some analogues of OPr on the formation of collagen in animals and on the urinary excretion of OPr will be studied.

Possibility of an abnormal ground substance (mucopolysaccharide) metabolism in Marfan's or Erlos Danlos syndromes will be investigated.

Part B included Yes ☐ No ☒

Form No. CRP-2
Oct. 1957

Serial No. NHI-246
1. Laboratory of Clinical Biochemistry
2. None
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Biogenesis and Metabolism of Aromatic Compounds

Principal Investigator: Dr. Chozo Mitoma

Other Investigators: Mr. Herbert S. Posner and Dr. Sidney Udenfriend

Cooperating Units:
1. Dr. A. Sjoerdsma, Clinic of General Medicine and Experimental Therapeutics, NHI
2. Dr. Frederick Leonard, Geigy Pharmaceutical Company.

Man Years (calendar year 1957):
Total: 3
Professional: 2
Other: 1

Patient Days (calendar year 1957):
None

Project Description:

Studies on phenylpyruvic oligophrenia:

To determine the origin of urinary o-hydroxyphenylacetic acid labelled compounds were administered to phenylketonuric patients. Phenylalanine-C^{14} gave rise to labelled o-hydroxyphenylacetate in the urine while phenylacetate-C^{14} did not.

Attempts to demonstrate the presence of o-tyramine, the suspected intermediate, in the blood and urine of phenylpyruvic patients, after loading with phenylalanine (4 gm), were not successful.

Biogenesis of aromatic compounds:

The microsomal hydroxylating system forms 3-hydroxyquinoline and a dihydrodiol type compound from quinoline. Although it would appear likely that the dihydrodiol is a precursor of 3-hydroxyquinoline no clear cut evidence of this has been obtained.

The same enzyme system catalyzes the formation of naphthalene dihydrodiol from naphthalene. Some indirect evidence has been obtained to show that the enzymes involved in the formation of this dihydrodiol differ from the hydroxylating system in some respects.

Serial No. NHI-246

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Calendar Year 1957

The aromatization of hexahydrobenzoic acid to form hippuric acid was studied using guinea pig liver mitochondria. The sequence of reactions was established as follows:

Hexahydrobenzoate + ATP + CoA ⟹ Hexahydrobenzoyl CoA

Hexahydrobenzoyl CoA ⟹ Benzoyl CoA

Benzoyl CoA + glycine ⟹ Hippuric acid

It appears that the fatty acid oxidase system is involved in the oxidation steps. Glycine or sarcosine stimulated the overall reaction by 300-400 per cent.

Iproniazid and phenylalanine will be administered simultaneously to see whether o-tyramine may be detected in phenylpyruvic patients.

Since quinic acid is found in many fruits and is known to be an important precursor of urinary hippuric acid, the enzyme system responsible for aromatizing this compound will be investigated. Also it is of interest to see how all 3 of the hydroxy groups on quinic acid are removed in the formation of benzoic acid.

Studies on aromatic hydroxylation will be conducted using tritiated substrates to see whether any information can be obtained on the mechanism of activation of the compound.

Part B included Yes [X] No [ ]

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Mitoma, C., Auld, R.M., and Udenfriend, S. On the Nature of Enzymatic Defect in Phenylpyruvic Oligophrenia. Proc. Soc. Exptl. Biol. and Med. 94: 634-635, 1957.

2. Mitoma, C., Posner, H.S., Bogdanski, D.F., and Udenfriend, S. Biochemical and Pharmacological Studies on o-Tyrosine and Its meta- and para-Analogues. A suggestion concerning phenylketonuria. J. Pharmacol. and Exptl. Therap. 120: 188-194, 1957.

3. Waalkes, T.P., and Udenfriend, S. A Fluorometric Method for the Estimation of Tyrosine in Plasma and Tissues. J. Lab. and Clin. Med. 50: 733-736, 1957.

4. Mitoma, C., Posner, H.S., and Leonard, F. Aromatization of Hexahydrobenzoic Acid by Mammalian Liver Mitochondria. Biochim. et Biophys. Acta. In Press.

Honors and Awards relating to this project:

None

Form No. ORF-2
Oct. 1957

Serial No. NHI-247
1. Laboratory of Clinical Biochemistry
2. None
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Biosynthesis and Metabolism of γ-Guanidinobutyric Acid and γ-Aminobutyric Acid

Principal Investigator: Dr. John J. Pisano

Other Investigators: Dr. Sidney Udenfriend and Dr. Chozo Mitoma

Cooperating Units:
1. Drs. Stephen Kuffler and Charles Edwards, Johns Hopkins University Medical School.
2. Dr. Harry Grundfest, Columbia University Medical School.
3. Schering Research Laboratories, Bloomfield, N.J.

Man Years (calendar year 1957):
Total: 5
Professional: 3
Other: 2

Patient Days (calendar year 1957):
None

Project Description:

It has been shown that γ-guanidinobutyric acid is synthesized by a transamidination reaction involving γ-aminobutyric acid and an amidine donor such as arginine. Of a variety of tissues tested, only kidney is active. The reaction is reversible. γ-Aminobutyric acid is about 11% as active as glycine. A number of structurally related compounds were tested and shown to be much less active than γ-aminobutyric acid, or totally inactive. In the rat, transamidinase appears to be located only in kidney, and mitochondria have about as much activity as the soluble cellular fraction.

γ-Guanidinobutyric acid has weak γ-aminobutyric acid activity in inhibiting the crayfish stretch receptor but has marked convulsant activity in mammals when appropriately administered.

Experiments are in progress to (1) determine the biological activity of γ-guanidinobutyric acid and (2) to explore possible new pathways of γ-aminobutyric acid metabolism particularly in relation to γ-amino-β-hydroxy-butyric acid and carnitine.

Part B included Yes [X] No [ ]

Serial No. NHI-247

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Pisano, J.J., Mitoma, C., and Udenfriend, S. Biosynthesis of γ-Guanidinobutyric Acid from γ-Aminobutyric Acid and Arginine. Nature, 180: 1125-1126, 1957.

Honors and Awards relating to this project:

None

Form No. ORP-2
Oct. 1957

Serial No. NHI-240
1. Laboratory of Clinical Biochemistry
2. None
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on Adrenaline, Noradrenaline and Related Catechol Compounds

Principal Investigator: Dr. Sidney Udenfriend

Other Investigators: Mr. Lemuel C. Leeper

Cooperating Units:
1. Dr. J.A.R. Mead, Laboratory of Chemical Pharmacology, NHI
2. Dr. A. Sjoerdsma, Clinic of General Medicine and Experimental Therapeutics, NHI

Man Years (calendar year 1957):
Total: 2
Professional: 1
Other: 1

Patient Days (calendar year 1957):
None

Project Description:

Studies on the metabolism of adrenaline and noradrenaline by soluble monoamine oxidase have permitted the isolation and characterization of the intermediate aldehyde, 3,4-dihydroxyphenylglyoxal, and the acid, 3,4-dihydroxymandelic acid.

It was also shown that tissues can convert a large number of catechols to their 3-methoxy analogues. Noradrenaline is thus converted to 3-methoxy-noradrenaline (refer also to report by Dr. Axelrod, NIMH). Methoxy noradrenaline is a better substrate of monoamine oxidase than is noradrenaline itself. Since O-methylation can occur on the amine as well as on the final acid it remains to be determined whether, *in vivo*, oxidative deamination precedes O-methylation or vice versa.

Biochemical studies on patients with pheochromocytoma and on the tumors themselves, have been continued. Additional C^{14} studies on urinary noradrenaline in these patients, confirm the rapid turnover of this catecholamine in the tumor. The isolated tumor has also been shown to be a rich source of the enzyme which hydroxylates 3,4-dihydroxyphenylethylamine to noradrenaline.

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Individual Project Report
Calendar Year 1957

Further studies will be carried out on N-methylation and O-methylation of catecholamines in patients and in experimental animals. Studies on patients with pheochromocytoma will be completed.

The mechanism of the side-chain oxidation leading from dihydroxyphenylethylamine to noradrenaline will be investigated.

Studies on inter-conversion of noradrenaline and adrenaline in vitro will be carried out.

Part B included Yes [X] No [ ]

Serial No. NHI-248

PHS-NIH
Individual Project Report
Calendar Year 1957

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Rosenfeld, G., Leeper, L.C., and Udenfriend, S. Biosynthesis of Norepinephrine and Epinephrine by the Isolated Perfused Calf Adrenal. Arch. Biochem. Biophys. In Press.

Honors and Awards relating to this project:

None

Form No. CRP-2
Oct. 1957

Serial No. NHI-249
1. Laboratory of Clinical Biochemistry
2. None
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on the Biosynthesis of Indoleacetic Acid (IAA)

Principal Investigator: Dr. Herbert Weissbach

Other Investigators: None

Cooperating Units:
1. Dr. A. Sjoerdsma, Clinic of General Medicine and Experimental Therapeutics, NHI

Man Years (calendar year 1957):
Total: 1
Professional: 1
Other: None

Patient Days (calendar year 1957):
None

Project Description:

With the help of a specific chemical assay, developed in this laboratory, for IAA in urine it has been possible to study the formation of this acid from tryptophan. A large series of 24 hour urines were assayed with 5 cases of elevated IAA excretion. There was no apparent correlation between the clinical manifestations and the elevated IAA excretion values.

Gut sterilization in normal subjects decreased the IAA value by 30%, indicating that the major part of the IAA formed is made by the tissues. In one patient with an IAA excretion of 200 mg/day the use of antibiotics caused a 70% drop in IAA excretion. Bacteria isolated from stool specimens from this patient, when grown on a tryptophan media, formed appreciable quantities of IAA. The addition of α-ketoglutarate enhanced this conversion, suggesting that transamination was involved. However, bacterial formation of tryptamine was also demonstrated suggesting that some IAA may arise from the amine.

Tryptophan tolerance experiments in humans showed that the ingestion of tryptophan caused a large rise (6 fold) in the urinary excretion of IAA for several hours after the ingestion of the amino acid. No increase in 5HIAA excretion was observed.

In vitro studies using animal tissues have shown the presence of an enzyme system that can convert tryptophan to IAA. No evidence for tryptamine formation was observed which suggested that indolepyruvic acid (IPA)

was the intermediate in this reaction. The enzyme system present in guinea pig liver high speed supernatant fraction was purified 6 fold. The reaction was inhibited by carbonyl trapping agents supporting IPA as an intermediate in the reaction. Tyrosine was also active in this system, with the resulting formation of p-hydroxyphenylacetic acid. Whether transamination or oxidative deamination is involved has not been definitely ascertained but the failure of dialysis to inactivate and of α-ketoglutarate and pyridoxal phosphate to stimulate suggest an oxidative mechanism.

Studies will be continued on the in vitro mechanisms involved in converting tryptophan to IAA. Applications to clinical material will be considered.

A method for measuring tryptamine in urine and tissues will be developed and applied to patients to determine factors involved in its formation, diet, intestinal flora, etc. Attempts will be made to isolate the intestinal organism which decarboxylates tryptophan to tryptamine, and to purify and study the decarboxylase.

Part B included Yes ☐ No ☒

Form No. ORP-2
Oct. 1957

Serial No. NHI-250
1. Laboratory of Clinical Biochemistry
2. None
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1957

Part A.

Project Title: Studies on Serotonin Metabolism

Principal Investigator: Dr. Herbert Weissbach

Other Investigators: Mrs. Betty G. Redfield and Dr. Sidney Udenfriend

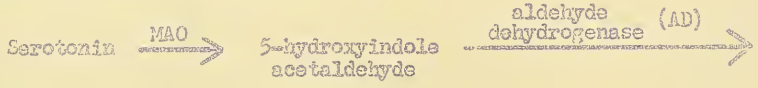
Cooperating Units:
1. Dr. B. Witkop, Laboratory of Chemistry, NIAMD-58
2. Dr. T.P. Waalkes, Clinic of General Medicine and Experimental Therapeutics, NHI
3. Dr. G. Glenner, Laboratory of Pathology and Histochemistry, NIAMD-65
4. Dr. S. Hess, Laboratory of Chemical Pharmacology, NHI

Man Years (calendar year 1957):
Total: 3.25
Professional: 2
Other: 1.25

Patient Days (calendar year 1957):
None

Project Description:

The major route of serotonin metabolism has been shown to be catalyzed by monoamine oxidase (MAO). Studies on this enzyme, in the past, have been limited since all attempts to solubilize this mitochondrial protein were unsuccessful. A MAO present in the soluble fraction of the cell has been found, which although weaker in activity than the mitochondrial enzyme, was more suitable for research studies. The enzyme has been purified over 20 fold from guinea pig liver, and has been shown to behave similarly to the mitochondrial MAO. The conversion of serotonin to 5-hydroxyindoleacetic acid (5HIAA) has been studied in detail using this soluble MAO. The complete conversion is presented below:



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MAO has been separated from AD and the aldehyde intermediate has been demonstrated directly. In the presence of the complete system (MAO, AD, and DPN) one µM of 5HIAA was formed for every µM of serotonin destroyed. The reaction could, therefore, be easily followed by measuring reduction of DPN. This soluble enzyme has also been used in studying the metabolism of the catecholamines and their methoxy derivatives (see report on Catecholamines).

Other studies on MAO have shown that tetrazolium salts can be used to measure MAO activity when using an indole amine as substrate. Dye reduction was shown to be dependent on the formation of an indole carbonyl compound.

$$\text{Indole amine} \xrightarrow{\text{MAO}} \text{indole carbonyl compound} \xrightarrow[\text{and tetrazolium reduction}]{\text{further oxidation}}$$

This work was done in collaboration with Dr. George Glenner who studied the use of tetrazolium salts in measuring MAO histochemically.

Further studies were performed on the effect of marsilid on serotonin metabolism *in vivo*. This drug, which is a potent inhibitor of MAO *in vitro*, was shown previously not to affect the disappearance of injected serotonin in whole mice. It was shown that acute doses of marsilid only raised serotonin levels in the brain. These studies have been extended, and it has been shown that although marsilid had virtually no effect on the rate of serotonin disappearance in the whole animal, this change markedly reduced the amount of 5HIAA formed after a given dose of serotonin. These results are summarized below:

Marsilid treated animal ⟶ Serotonin destruction but no 5HIAA found

Untreated animal ⟶ Serotonin destruction with 5HIAA found

Two possibilities are evident. Either there is another pathway of serotonin metabolism *in vivo*, or marsilid inhibits the conversion of 5-hydroxyindoleacetaldehyde to 5HIAA. This problem is still under investigation.

The importance of marsilid as an inhibitor of MAO has initiated a study on the metabolism of this drug. A method for the colorimetric estimation of marsilid in tissues has been developed. This drug is apparently metabolized *in vivo*, although only slight metabolism was observed in tissue slices. Evidence has been obtained that a large proportion (20%) of the marsilid destroyed by liver slices is converted to isonicotinic acid. These studies are now being extended in collaboration with Dr. S. Hess (Laboratory of Chemical Pharmacology).

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A large number of indole analogues, supplied by Dr. B. Witkop (NIAMD), have been tested as inhibitors for MAO and 5-hydroxytryptophan (5HTP) decarboxylase. 2-Oxytryptamine was found to be a potent in vitro inhibitor of MAO, although little in vivo action was seen. Its inhibition could be reversed by the addition of excess substrate. Although many other analogues were inhibitors of MAO, only one in vitro inhibitor of 5HTP decarboxylase was found. This compound, α-hydroxytryptophan, was not active in vivo.

The role that serotonin plays in anaphylaxis was investigated with Dr. T.P. Waalkes, Clinic of General Medicine. It was found that both serotonin and histamine are released into the plasma during anaphylaxis in the rabbit. Studies with reserpine showed that although histamine was released from both rabbit tissue and platelets, the serotonin released during the anaphylactic reaction came primarily from the blood platelets. A simplified method for the estimation of serotonin in tissues was developed, along with a combined method for the estimation of both serotonin and histamine on the same tissue aliquot.

Further purification of MAO will be carried on in order to determine the cofactor involved and to understand the mechanism of the reaction. Studies will also be carried on to determine the mechanism of MAO inhibition by marsilid and other agents. These will include studies on the metabolism of marsilid in vivo and in vitro. Additional studies on factors responsible for release of serotonin, anaphylaxis, etc., will be continued.

Part B included Yes [X] No [ ]

Serial No. [illegible]

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Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Weissbach, H., Bogdanski, D.F., Redfield, B.G., and Udenfriend, S. Studies of the Effect of Vitamin B_6 on 5-Hydroxytryptamine (Serotonin) Formation. J. Biol. Chem. 227: 617-624, 1957.

2. Udenfriend, S., Weissbach, H., and Bogdanski, D.F. Effect of Imipramine on Serotonin Metabolism in vivo. J. Pharmacol. and Exptl. Therap. 120: 255-260, 1957.

3. Waalkes, T.P., Weissbach, H., Bozicevich, J., and Udenfriend, S. Serotonin and Histamine Release During Anaphylaxis in the Rabbit. Clin. Invest. 36: 1115-1120, 1957.

4. Waalkes, T.P., Weissbach, H., Bozicevich, J., and Udenfriend, S. Further Studies on Release of Serotonin and Histamine During [illegible] in the Rabbit. Proc. Soc. Exptl. Biol. and Med. 95: 479-[illegible], 1957.

5. Weissbach, H., Redfield, B.G., Glenner, G., and Mitoma, C. Tetrazolium Reduction as a Measure of Monoamine Oxidase Activity in vitro. J. Histochem. and Cytochem. 5: 601-605, 1957.

6. Davidson, J.D., Sjoerdsma, A., Loomis, L.N., and Udenfriend, S. Studies with the Serotonin Precursor, 5-Hydroxytryptophan, in [illegible] mental Animals and Man. J. Clin. Invest. 36: 1594-1599, 1957.

7. Weissbach, H., Redfield, B.G., and Udenfriend, S. Soluble Monoamine Oxidase: Its Properties and Actions on Serotonin. J. Biol. Chem. In Press.

8. Weissbach, H., Bogdanski, D.F., and Udenfriend, S. Binding of [illegible] and Other Amines by Blood Platelets. Arch. Biochem. Biophys. [illegible]

9. Weissbach, H., Waalkes, T.P., and Udenfriend, S. A Simplified [illegible] for Measuring Serotonin in Tissues; Simultaneous Assay of Both [illegible] and Histamine. J. Biol. Chem. In Press.

10. Lovenberg, W., Weissbach, H., Redfield, B.G., Udenfriend, S., and [illegible] Oxindole Analogues of (5-Hydroxy) Tryptamine and Tryptophan, Inhibitors of the Biosynthesis and Breakdown of Serotonin. J. Am. [illegible] Soc. In Press.

Honors and Awards relating to this project:

None

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PHS-NIH
NATIONAL HEART INSTITUTE

Summary Budget Data
Laboratory of Gerontology

Estimated Obligations for FY 1958

Total:	$425,000	
Direct:		$425,000
Reimbursements:		0

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

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 (1/1/57 - 6/30/57)
Robert I. Gregerman (1/3 time)

Other Investigators: N. W. Shock
Marvin J. Yiengst

Technical Assistance: Samuel E. Crowder (1/2 time)
Margaret Sellmayer (1/2 time)

c. Progress During Past Twelve Months: Studies from this and other laboratories in the past have indicated a progressive decline in basal metabolic rate (BMR) with advancing years which was thought to represent a decrease in thyroid activity. However, as this laboratory later showed, the age decrease in BMR is explainable on the basis of a concomitant decrease in functioning protoplasmic mass. In view of these findings, and because of the inadequate and conflicting nature of the age-wise studies of thyroid function, studies measuring thyroid activity more directly were initiated in this laboratory in 1955. The results of responses to the administration of thyroid stimulating hormone were reported last year. This report deals with the work on the uptake of radioiodide by the thyroid and the level of circulating thyroid hormone (protein bound iodine). Gathering of data for this study ended in December 1956. Data processing is in progress. It would appear, however, that no significant age-wise change in thyroidal accumulation of radio-iodide is demonstrable in a large series of subjects (about 140) over the age of fifty years. Similarly the range of serum protein bound iodine is not altered with increasing age. Studies on age changes in plasma levels of vitamin B_{12} have been completed and the results published.

d. Direction of Current Research: It is expected that data processing will be completed within the next six months, and with this the study itself will be complete.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals provides space and all utilities.

4. Publications and Awards:

1. Shock, N. W.: Age changes in some physiologic processes. Geriatrics, 12: (1), 40-48, Jan. 1957.

2. Gaffney, G. W., A. Horonick, K. Okuda, P. Meier, B. F. Chow and N. W. Shock: Vitamin B_{12} serum concentrations in 528 apparently healthy human subjects of ages 12-94. J. Geront., 12: (1), 32-38, Jan. 1957.

3. Silverstone, F. A., M. Brandfonbrener, N. W. Shock, and M. J. Yiengst: Age differences in the intravenous glucose tolerance tests and the response to insulin. J. clin. Invest., 36: (3), 504-514, March 1957.

4. Shorr, E. (Moderator), D. Laszlo, N. W. Shock, and G. D. Whedon (Panelists): Panel discussion on steoporosis. J. Amer. geriat. Soc., 5: (4), 363-384, April 1957.

5. Baker, S. P.: Heparin-activated clearing factor standardized test, agewise application, and clinical observations. Circulation, 15: (6), 889-896, June 1957.

Prepared by Robert I. Gregerman
October 28, 1957

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

1. a. Title: Thyroid function and age - thyroxine degradation study.

b. Principal Investigator: Robert I. Gregerman (1/3 time)

Other Investigator: Marvin J. Yiengst

Technical Assistance: Samuel E. Crowder (1/2 time)
Margaret Sellmayer (1/2 time)
Raymond Flath (1/5 time)

c. Progress During Past Twelve Months: The recent age-wise studies of thyroidal radio-iodide uptake and serum protein bound iodine (PBI) levels from this laboratory show no striking age-wise alteration in these parameters of thyroid function. Recent studies in young and senescent rats have shown a small decrease in thyroidal iodide uptake and no change in serum PBI levels with senescence. Estimates in rats of the secretion rates of thyroid hormone (thyroxine) as determined by the goiter prevention technique show, however, a striking fall in the rate of thyroxine secretion in the old animals. These results imply, since the serum PBI does not change, a slowed rate of metabolic removal of thyroxine and an increased sensitivity of the old animals to the hormone. The latter has actually been demonstrated.

It is possible that neither the thyroidal radio-iodide uptake nor serum PBI accurately reflects thyroid function within the clinical enthyroid range. In the last analysis, the function of the thyroid gland can only be measured by determining the absolute quantity of thyroid hormone released from the gland per unit time.

Others have demonstrated in man that the steady state thyroxine synthesis equals thyroxine degradation. By measuring the latter therefore, one has a true index of thyroid function. The method involves administration of a tracer quantity of radio-thyroxine and calculation of the thyroxine degraded per unit time from its virtual volume of distribution, rate of removal from the plasma, and plasma PBI.

It is the purpose of this study to define in man the extent of age-wise alterations in thyroxine secretion and peripheral metabolism already suggested by animal experiments.

Since February 1957 radio thyroxine distribution volumes and degradation rates have been determined in fifty male subjects. Of these 28 have had two determinations. Ages range from 50 to 80 years. Analysis of the data to date indicates a progressive slowing of the rate of thyroxine turnover with increasing age, and a decrease in its virtual volume of distribution. As a result thyroxine degraded falls from a mean of 62 micrograms to 39 micrograms per day for the 50 year and 80 year old groups respectively. This result is highly statistically significant (p less than 0.001).

This work demonstrates beyond any doubt that the thyroidal iodide uptake and serum PBI levels are insufficient indices for the range of normal thyroid function, and shows a striking yet subtle age-wise alteration of an important metabolic function.

d. Direction of Current Research: Within the next six month period it is hoped to complete this study. A number of young adults are to be included, and determinations on a few more elderly subjects will be made to give 15 individuals in each age decade. A similar method may be applied to study of the same phenomenon in the rat. Ultimately, it may prove possible to establish the mechanism of the age-wise slowing of thyroxine turnover in an examination of the recently described enzyme system responsible for its metabolic degradation.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Robert I. Gregerman
October 28, 1957

Serial No.: NHI-253

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

1. a. Title: Age changes in the chemical composition of various tissues of the rat.

b. Principal Investigator: Marvin J. Yiengst (1/2 time)

Other Investigators: Charles H. Barrows
N. W. Shock

Technical Assistance: Ramona Dorcas
Janet Ellis (1/2 time)
James Tucker (1/4 time)
Patricia Knell (temporary summer employment - chemistry student - June 17 - September 10, 1957)

c. Progress During Past Twelve Months: The objectives of this project are to investigate age changes in chemical composition of tissues. The principal aim of the project is the development of a suitable means for the estimation of cell mass. An additional goal is the age change in protein and amino acid composition of serum and tissues.

Standard methods of chemical analysis are used for the determination of tissue components such as electrolytes, nitrogen, fat and water in organs and tissues of rats. Paper electrophoresis and chromatography are among the methods used for the studies of proteins and amino acids.

In a previous project report it was shown that the chemical composition of muscle indicated a 5% loss of cell mass in male rats due to aging and a smaller loss of 3% in old female animals. These changes, since they are based on wet tissue weight, do not answer the question as to the degree of change in total muscle mass. The additional information was obtained from weights of excised gastrocnemii and anterior tibial muscles in young and old rats. The gastrocnemius muscle weight of old males was 17% lower than that of young males. The anterior tibial group showed an 8% age decrease in the same animals. Old females showed a 9% decrease throughout. With one exception (anterior tibial group of old females) the muscle weights decreased at a greater rate than did body weight. In males, the gastrocnemius, when expressed as percent of body weight showed a 7% decrease ($P = .02$).

Results to date indicate that in male rats, mass of muscle (24 months) is about 12% lower in old animals than in young adults (12 months) when expressed as percent of total body weight. In females these changes are about one-half as great. Kidney tissue (cortex) of old animals shows a 5% decline in cell mass per unit of wet weight when based on potassium content and an increase in total organ weight which is sufficient to offset this loss. No changes of any significance in chemical composition were observed in brain or cardiac muscle.

d. Direction of Current Research: Work currently in progress is directed toward age changes in protein and amino acid composition of serum and tissues.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Marvin J. Yiengst
October 28, 1957

Serial No.: NHI-255

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

1. a. Title: Age changes in cellular and tissue biochemistry.

b. Principal Investigator: Charles H. Barrows

Other Investigators: N. W. Shock
Marvin J. Yiengst

Technical Assistance: Lois Roeder
Mary J. Blakely
Nancy Smith (Summer student 6/17/57 to 9/17/57)
William Smith (3/4 time)
James Tucker (1/2 time)

c. Progress During Past Twelve Months: The objective of this project is to examine various tissues of rats for age changes in the amount of protoplasm and to determine the metabolic characteristics of the existing protoplasm.

These experiments have been carried out on whole homogenates, made up either in distilled water or isotonic sucrose, and mitochondria prepared from various tissues of 12-14 month and 24-27 month old rats. The animals were bred and maintained in our laboratory quarters. Established methods have been employed for the determination of specific enzyme systems and of various tissue components such as DNA, RNA and protein N.

Recent data again demonstrate that the agewise decrease in the concentration of succinoxidase (11%, P = .01) of whole homogenates based on the wet weight of kidney tissue exceeds the loss of protoplasm (3%, not sifnificant) as measured by protein nitrogen. This decrement is apparently manifested by an agewise decreased rate of oxidation (11%, P = .01) and phosphorus esterified (11%, P = .01) when the phosphorylation coupled to the oxidation of succinate by whole homogenate of kidney is examined. However the efficiency of this metabolic process as measured by the P/O ratios is unaffected by age. Since no agewise change in the succinoxidase activity of mitochondria (based on protein nitrogen) was demonstrated, the reduced enzymic activity per unit of kidney protoplasm may be explained by a loss of mitochondria from the tissue. No agewise changes in these measurements were observed when liver tissue was examined. Furthermore the data fail to demonstrate an agewise change in the oxidative phosphorylation of mitochondria (based

on protein nitrogen) isolated from this tissue. Thus no evidence is available to indicate a change in the metabolic activity of mitochondria obtained from the tissues of old animals, although a loss of these particulates from the kidney tissue of old rats is inferred.

d. Direction of Current Research: Additional enzymes will be surveyed for age changes. Enzymes will be chosen in part to test the hypothesis of the existence of a preferential synthesis of certain enzymes over others by various tissues. In addition the ability of the tissues of rats of different ages to utilize the energy produced during oxidative phosphorylation to carry out other metabolic activities such as the conversion of PAS to PAH will be investigated. Finally investigations of age differences in the quantitative aspect of tissue protein synthesis will be continued using the depletion-repletion method.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards:

1. Barrows, C. H., and B. L. Chow: Studies on enzymes in arterial tissue, Chap. in: A. I. Lansing (Editor), The Arterial Wall, Its Aging, Structure and Chemistry. (In press).

Prepared by Charles H. Barrows
October 28, 1957

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

1. a. Title: Adaptive enzymes and age

b. Principal Investigator: Robert I. Gregerman (1/3 time)

Technical Assistance: William Smith (1/4 time)
James Tucker (1/4 time)

c. Progress During Past Twelve Months: In July 1957 measurements were begun of the ability of senescent rats to perform specific protein syntheses reflecting metabolic adaptations. Several mammalian liver enzymes are known to undergo "adaptation" under influence of several stimuli. In this phenomenon non-specific stress, adrenal hormones, and the enzyme's specific substrate itself are capable of causing an increase in tissue enzyme level which has been shown to be a reflection of rapid protein synthesis. Evidence exists for the presence of separate mechanisms in the adrenal and substrate induced adaptations which differ quantitatively and qualitatively.

Threonine dehydrase, tryptophane peroxidase, and tyrosine transaminase were chosen for adaptation studies. The assay of threonine dehydrase according to published methods was found to be invalid for purposes of comparing groups of animals. Tryptophane peroxidase (TPO) assay was standardized and found to be a reliable system. Standardization of the tyrosine transaminase (TT) assay is being attempted.

So far neither basal enzyme levels nor hormone adaptation have been studied for the TPO or TT systems. However, the ability of the senescent (24 to 26 month old) rat to perform TPO substrate induced adaptation has been compared to that of the adult (12 month) animal. As measured by this index, the liver of the senescent rat shows no significant change in ability to perform rapid, short-term, protein synthesis.

d. Direction of Current Research: In the next few months we hope to study the hormone induced adaptation of tryptophane peroxidase and tyrosine transaminase, and substrate induction of the latter if possible. Basal levels of these enzyme will also be determined.

As tests of the hypothesis that senescence is accompanied by a biochemically demonstrable diminution in metabolic

responsiveness, it is hoped that these observations will lead to a better understanding of the metabolic phenomena associated with aging.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Robert I. Gregerman
October 28, 1957

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

1. a. Title: Cardiovascular hemodynamics III. The peripheral circulation in man.

   b. Principal Investigator: Milton Landowne

   c. Progress During Past Twelve Months:

   Objectives: To study the circulation in the extremities of living human subjects, with respect to changes with age, disorders in function, and to increase our understanding of factors governing the blood flow to these tissues.

   Methods Employed: Skin temperature is used as an index of circulation to the skin, and venous occlusion plethysmography as a measure of blood flow to an extremity. Studies are carried out on rate of skin cooling under controled thermal environment, and on the resting and 'vasodilated' flow to the foot. Rectal temperature is recorded as an index of "core" temperature.

   Clinical Material: Subjects are from our research ward, other hospital wards, and members of the staff.

   (Project inactive during 1957)

   d. Direction of Current Research: Inactive

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Milton Landowne
October 28, 1957

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

1. a. Title: Cardiovascular Hemodynamics. II. Cardiac performance in man.

b. Principal Investigators: Milton Landowne (1/2 time)
Joseph Falzone (1/4 time)
Theodore Reiff (1/5 time)

Technical Assistance: Raymond Flath (1/5 time)
Edna Phillips (2/3 time)
Jesse Yaffe (1/2 time)

c. Progress During Past Twelve Months:

Objectives: To study the performance and functional limitations of the living human heart as affected by age and disorders of the circulation.

Methods Employed: (A) Cardiac output is measured by dye dilution technique at rest and during graded exercise at two levels of exercise. Femoral arterial pressure is measured by intra-arterial needle and capacitance manometer. Arterial blood gases are determined manometrically. Ventilation, O_2 uptake and CO_2 production are determined to provide measures of ventilation and respiratory gas exchange. Calculations of cardiac output, work 'power' and peripheral resistance are considered in relation to rest, exercise, ventilation, O_2 uptake and work performed.

Clinical Material: Subjects are obtained from our 60 bed ward the other wards of Baltimore City Hospitals and the Hospital Infirmary.

(B) The response of 2 body sites to an applied sinusoidal force of essentially constant amplitude and over a selected frequency range are compared for subjects of different age and ballistocardiographic patterns.

Additional experiments have been performed and the results of 35 runs on 33 subjects reviewed. Information in the previous report is generally substantiated and preliminary agewise orientation of the series suggests the following:

At rest, the group displays a general agewise decrease in cardiac output, left ventricular work and power; increase in

A-V difference, mean transit time and peripheral resistance; appearing to support the observations previously made. These have been not tested for statistical significance. We have too few subjects below 50 and over 80 for critical agewise analysis.

After 3 minutes of moderate arm exercise the group are less homogeneous in performance, with respect to the above items, than at rest. This is true in age decade categories. Nevertheless, in the exercising subject a general agewise decrease in output, work and power is still suggested, with increase in mean transit time and more striking increase in A-V difference and in NWRI, (a measure of peripheral resistance which is described below). Stroke work estimates appear to furnish the greatest individualization or dispersion.

The increased oxygen uptake during exercise usually exceeds that contained in the additional blood pumped by the heart. There is, therefore, some additional diversion of blood from other organs into the exercising area. A rough minimal measure of this diversion is provided by estimating the vascular resistance in the non-exercising region. This has been called the "non-working resistance index" (NWRI). A high value suggests a marked peripheral vascular adjustment to exercise conditions; a low value suggests a more optimal circulatory deployment.

The following circulatory concept has been formulated: Just as at rest, where the left ventricular stroke work or power expresses an index of cardiac performance and the peripheral resistance furnishes an index of vascular state; so during steady state exercise, left ventricular stroke work and the "non-working resistance index" may serve as indices of the adjustment of the heart, and of the periphery, respectively, to the added requirements of exercise. Agewise and individualwise consideration of these two indices permit the identification of different degrees of central and peripheral adjustment. Support of exercise by diversion of flow from non-exercising areas not only compromises the flow reserve of these areas but the associated increase in resistance results in a relatively higher arterial pressure and thus imposes a less favorable work load on the heart. While only the general nature of the circulatory response to exercise may be indicated in this manner, these indices are likely to be more meaningful for this purpose than are the commonly used 'basic' data from which they may be derived (blood pressure, heart rate, cardiac output, arterial oxygen).

Consistant records are obtainable indicating the response of the body to externally applied forces. The apparatus has been further modified and experiments can be performed with greater reliability and ease. Mr. Plath has analyzed records of 11 subjects with an age range of 12-81 years and a weight range of

90-300 pounds. Individually characteristic differences in phase shift appear but these are not of large magnitude compared to the general frequency dependence of phase. Individual differences in amplitude of motion are also less striking than the general frequency dependence of amplitude. No conclusions have been reached.

d. Direction of Current Research: Further information is desirable. Ballistic pattern analysis requires completion.

2. Patient Days: 159

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards:
   1. Landowne, M.: Methods and limitations in the study of human organ system function. Ciba Foundation Colloquim on Methodology of the Study of aging, 1957.

Prepared by Milton Landowne
October 28, 1957

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

1. a. Title: Cardiovascular hemodynamics I. Arterial performance in man.

b. Principal Investigator: Milton Landowne (1/2 time)

Technical Assistance: Theodore Reiff (1/10 time)
Jesse Yaffa (1/2 time)
Raymond Flath (1/5 time)

c. Progress During Past Twelve Months:

Objectives: To investigate the functioning of the arterial circulation in living human subjects in the following manner:

a. To develop and to test critically methods of studying the dynamic behavior of large and medium sized arteries in situ.
b. Using these, and existing methods, to describe individual, and agewise differences in arterial function in ostensibly normal subjects of mature age.
c. To characterize evidences of pathophysiological performance in arteriosclerosis, hypertension and other circulatory disorders.

Methods Employed: The way in which arteries function to transmit pressure and flow of blood to the small vessels is determined by viscous and elastic properties of the arterial wall. We are using 3 methods of investigating these properties and arterial performance. a. Small transient or sustained waves of pressure are created in arteries by a method developed in this laboratory. b. The pressure pulse produced by the heart is subjected to harmonic analysis. c. The decline of blood pressure during diastole (between heart beats) is compared to the theoretical behavior of models of increasing complexity.

Pertinent intra-arterial pressures are detected at various positions within the arterial tree by intra-arterial needles and special catheters attached to capacitance type sensing devices, and recorded, with sensitive and accurate equipment.

The speed of pressure propagation, and its distortions, damping, resonance and reflection are then computed at varying pressures, and for waves of different frequencies. These data provide indices of elastic and viscous behavior.

Graphic and mathematical analysis of pressure curves in diastole provide information about the validity and practicality of simplifying assumptions regarding the behavior of the arterial system.

Patient Material: Subjects from our 60 bed male ward, the Baltimore City Hospitals Infirmary and other wards of Baltimore City Hospitals.

Mathematical analysis of simultaneously recorded pressures at a series of paired intra aortic and arterial locations indicates that in the propagation of pressure in the human aorta factors of damping and reflection must be considered. Reflection is significant for low frequency components. These components represent the bulk of the pressure energy, and this fact precludes the accurate interpretation or use of pulse wave (phase) velocity. Damping is significant for high frequency components, therefore reflections here are less evident, but as the amplitude of these components are initially small, they contribute to the detail form rather more than to the major part of the propagated energy.

The use of femoral pressure to obtain systolic duration aortic mean systolic pressure and aortic mean pressure has been compared with the previous use of brachial pressure. Slight consistent differences are noted. For accurate interpretation of cardiovascular hemodynamics a more extensive direct comparison of femoral and brachial pressures with proximal aortic pressures would be desirable.

d. Direction of Current Research: These studies are reaching their conclusion. Further exploration should involve models, and isolated preparation, with firmly based mathematical theoretical and technical support. This would be more appropriately provided in a cardiovascular laboratory.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards:

1. Landowne, M.: Pulse Wave Velocity as an Index of arterial elastic characteristics in Tissue Elasticity. edited by J. W. Remington. Am. Physiol. Society 1957.
2. Landowne, M., and R. W. Stacy: A list of terms used to describe the mechanical properties of tissues. In: Tissue Elasticity edited by J. W. Remington. Am. Physiol. Society 1957.

3. Landowne, M.: A method using induced waves to study pressure propagation in human arteries. Circulation Research, (in press) November 1957.

Prepared by Milton Landowne
October 28, 1957

Serial No.: NMI-260

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

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 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 (1/3 time)

Other Investigators: Joseph A. Falzone
David Oursler

Technical Assistance: John B. Melvin (1/3 time)
Mae F. Griffin (1/2 time)

c Progress During Past Twelve Months: Studies of pulmonary and metabolic responses to the submaximal levels of exercise have been carried out in 81 subjects from 21 to 87 years of age. Significantly increased responses of both maximum ventilation rate and total excess ventilation with increase in age were tentatively attributed to moderate accumulation of lactic acid and low CO_2 elimination relative to ventilation, rather than to increased pulmonary dead space. Reduced maximum oxygen uptake seen in the oldest subjects during exercise was considered to be consistent with previously described reductions in maximal diffusing capacity. Moreover, the oldest subjects were shown to use more oxygen for a given amount of work than

the younger subjects. Only one half of the extra oxygen used by the oldest subjects could be accounted for by the increased cost of repaying the oxygen debt after work rather than during work. The cost of the work of the extra ventilation seen in the older subjects may account for a large part of the remaining half of the extra oxygen used by the older subjects.

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 ageing 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
October 28, 1957

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

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: Richard Myers (3/4 time)
Arthur H. Norris (1/3 time)

Other Investigators: Nathan W. Shock
Marvin J. Yiengst

Technical Assistance: Constantine J. Maneras

c. Progress During Past Twelve Months: Fat thickness measured from standard chest x-ray films was compared for 32 subjects 20 to 49 years of age and 47 subjects 50 to 89 years old. Average fat thickness increased from 7.9 mm. in the young subjects to 8.1 mm. in the old subjects despite a decrease in average body weight of 10 Kg. This suggests that fat free weight decreases more rapidly with age than total body weight.

Standardization of the helium displacement method of body volume measurement has been carried out. Standard volumes

can be determined from other standards within 2% of the actual volume.

d. Direction of Current Research: An integrated measurement program which includes selected indices of body size and composition will be initiated. Some techniques may be provided through cooperation of investigators outside the Public Health Service, while others are standardized procedures used in this laboratory. Data will be made 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, and
Dr. Stanley M. Garn, Fels Research Institute, Antioch College

4. Publications and Awards: None

Prepared by Arthur H. Norris
October 28, 1957

Serial No.: NHI-262

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

1. a. Title: Age changes in human performance.

This project is designed to study the effects of ageing 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 (1/3 time)
Joseph A. Falsone (1/4 time)

Other Investigators: Nathan W. Shock
Milton Landowne
Theodore R. Reiff

Technical Assistance: John B. Melvin (2/3 time)
Eleanor E. Howard
Mae F. Griffin (1/2 time)
Edna Phillips (1/3 time)
Elisabeth Benser (2/3 time - resigned May 31, 1957)
Dora Goldblatt (E.O.D. - August 1, 1957) (2/3 time)

c. Progress During Past Twelve Months: The studies of metabolic and cardiovascular responses to a high level of manual exercise have been continued. Of about 150 subjects screened by clinical examination and preliminary strength measurements, 82 have had measurements of ventilatory and metabolic responses to the high level of exercise. Just over 30 subjects have had combined estimates of ventilatory, metabolic and cardiovascular responses during exercise at the "maximal" level and a level of 540 Kg.M. at 135 Kg.M/min.

The older subjects were found to be less able to maintain a constant low work rate for a period of 4 minutes. The rate of increase in range of variation with increase in age was linear up to age 65. Extrapolation of this rate predicted the range of variation of 80 year old subjects who were not able to perform the high level of work but not the relatively decreased range of variation of the 80 year old subjects who were able to perform at the high level. Maximum work rate (Kg.M./min.) decreased linearly with age for short spurts of effort. The younger subjects were able to maintain higher outputs (Kg.M./min. at higher cranking rates (R.P.M.) than the old.

The reproducibility of the "maximal" work level from one day to another was good as judged by uniformity of work rate (expressed as per cent of maximum work rate) and the time it was maintained (time of plateau). The average time of plateau and per cent of maximum work rate were lower in basal subjects (0.85 min., 37%) than in resting subjects (1.00 min., 39%). Although the average total amount of work performed decreased from 950 Kg.M. in the 30 year old subjects to 400 Kg.M. in the 80 year old subjects, the average net mechanical efficiency remained between 10.2 and 11.3 per cent for the entire age range (30 through 80 years.).

No significant age differences appeared in average calculated arterial-venous differences or mixed venous levels for blood O_2 or CO_2 at rest or during either of the two exercise levels used The age range of these subjects was limited to 50 through 89 years.

Calculation of the "action" (force x displacement x time) of individual swings of an arm wagging exercise in six 20-30 year old subjects and six 80-89 year old subjects discloses a wide variability of this measure which has been suggested as an index of mechanical efficiency. The variability of "action" in the older subjects is greater than in the young. When "action" of the most powerful swings is plotted against amplitude of swing, a linear curve of increasing amplitude with increasing "action" is observed. In about one half of the subjects a point of inflection of the curve was observed at a displacement of about 1.50 radians. Beyond this point no increase in amplitude is observed for large increases in "action". In young subjects

there is a significant decrease in the average power per swing with increase in amplitude from 0.2 radians to 2.5 radians. The same relation holds for the older subjects, but is only demonstrable for the swings with the highest "actions" observed. This suggests that when the duration and amplitude of swings are large, a smaller amount of power is used in the simultaneous contraction of opposing muscles than when swings are short and rapid. This smaller power consumption might be associated with greater mechanical efficiency of large amplitude movements as compared with small amplitude movements when both are performed with maximum torque.

d. Direction of Current Research: The screening procedures and measurement of responses to "maximal" exercise will be continued. These measurements plus cardiac output determinations will be made on selected subjects. The limb movement studies will be continued with a comparison of the maximal actions and net mechanical efficiencies measured during a series of swings. Studies of timing of action potentials will be investigated further in young and old subjects.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals, and
Dr. Robert Ramsey, Medical College of Virginia
Assistance of: Frances M. Beran
Lorraine D. Ward

4. Publications and Awards:

1. Shock, N. W.: A Classified Bibliography of Gerontology and Geriatrics. Stanford University Press, Stanford, Calif., Supplement I 1957, xxviii, 525 pp.

2. Shock, N. W.: Trends in Gerontology. Stanford University Press, Stanford, California, 2nd Edition, 1957, viii, 214 pp.

Prepared by Arthur H. Norris
October 28, 1957

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

1. a. Title: Care of patients housed on Ward B-2 (60 beds) who are used in various studies at this post. Screening all male patients who are accepted for admission to the Baltimore City Hospitals Infirmary.

   Goals and Aims: All male admissions to the Infirmary are screened and given an exhaustive clinical medical workup in order that their general status be understood. The goal of this project is to provide suitable subjects for the various studies being carried on by non-clinical investigators and to provide medical care for these subjects.

   b. Principal Investigator: David A. Oursler (3/4 time)

   Technical Assistance: Clinical Laboratory of Hospital
   Emergency Laboratory studies by the investigator (5% time)

   c. Progress During Past Twelve Months: Assigned July 1, 1957 through January 1, 1958

   d. Direction of Current Research: Stated above

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by David A. Oursler
October 28, 1957

Serial No.: NHI-264

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

1. a. Title: Studies on optical properties of metabolizing systems

   b. Principal Investigator: Bernard L. Strehler (10% of time)

      Technical Assistance: Patricia Winter (5% of time)

   c. Progress During Past Twelve Months: This is a new project begun about September 1957.

   d. Direction of Current Research: Spectrophotometric determinations of steady state concentrations of respiratory intermediates have been undertaken on several in vivo systems including luminous bacteria at various temperatures. Using the difference spectrum techniques the oxidized minus reduced spectrum of A. fischeri has been measured and incidentally a finding of considerable interest has been made that there is a generalized wave length non specific change in transmission of these organisms when they become anaerobic. The nature of this effect and its significance to metabolism is being investigated.

2. Patients Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Bernard L. Strehler
October 28, 1957

Serial No.: NHI-265

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

1. a. Title: Studies on mathematical-physical expressions of population mortality.

   b. Principal Investigator: Bernard L. Strehler (10% of time)

   Other Investigator: Joseph Falzone

   c. Progress During Past Twelve Months: This is a new project begun about December 1956.

   d. Direction of Current Research: This research consists of an attempt to derive the Gompertz function describing age specific mortality rates from reasonable basic physical and biological assumptions. It was shown that one can account for the logarithmic increase in mortality with time exhibited by many species of animals on the basis of the following assumptions:
      1. Vitality-the ability to put out energy in forms useful for survival.
      2. Vitality decreases linearly with time.
      3. Death occurs when vitality is incapable of meeting a challenge to existence.
      4. Such challenges are distributed in magnitude according to the Maxwell-Boltzmann law.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: (Manuscript submitted for Proceedings of Gatlinburg Aging Conference).

Prepared by Bernard L. Strehler
October 28, 1957

Serial No.: NHI-265

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

1. a. Title: Studies on the effect of temperature on the aging process. II. Bacterial and enzyme "aging" studies.

   b. Principal Investigator: Bernard L. Strehler (20% of time)

      Technical Assistance: Patricia Winter (15% of time)
      Susanne Herman (20% of time)

   c. Progress During Past Twelve Months: This is a new project begun in September 1957.

   d. Direction of Current Research: The effect of temperature"shocks" on isolated enzymes and on intact metabolic sequences is being investigated utilizing principly the easily measured phenomenon of bioluminescence as test object. Luminescent reactions may be measured instantaneously with high accuracy and sensitivity. This makes them ideal tools for kinetic research. Moreover, since the "death" of bacteria results in loss of ability to luminesce, luminescence can be used as an index of the minimum viability of bacterial cultures. The exact quantitative relationship between luminescence and viability has not yet been determined. However, it appears that thermal shocks do not predispose bacteria to premature death when they are returned to lower temperatures. Additional studies of the effect of partial or reversible denaturation of enzyme systems is being pursued.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Bernard L. Strehler
October 28, 1957

Serial No.: NHI-267

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

1. a. Title: Studies on the effects of temperature on the aging process
I. Drosophila Studies

b. Principal Investigator: Bernard L. Strehler (15% of time)

Technical Assistance: Patricia Winter (since June 1957 - 70% of time)

c. Progress During Past Twelve Months: This is a new project begun in June 1957.

d. Direction of Current Research: Earlier work by Loeb, Northrup and Pearl has shown that temperature has a striking effect on the lifespan of poikilothermic animals. The present work has three major goals: (1) To ascertain more accurately the shape of the mortality curve with a constant density of flies (1 per bottle). (2) To ascertain whether the accellerated senility at high temperature is due to metabolic derangements or to thermal accidents. (3) To study the affect of temperature on metabolic patterns and steady states.

The general design of experiments directed toward the first goal is to incubate flies in individual cages from several hours after hatching until death. Various media with and without fungicidal agents have been used. Dead flies are removed daily including week-ends.

The second question will be answered by subjecting animals to transient thermal shocks (high temperatures) of varying intensity and then returning them to a more normal temperature. Similarly the animals will be subjected to low temperatures (near to freezing) to see whether this produces a rejuvenation. If temperature exerts its main influence by controlling the rate of denaturative reactions, a short period at high temperatures should produce animals of apparent greater age (much as a single dose of ionizing radiation does). If no affect on mortality is observed, then this thesis is highly unlikely. The converse inferences could be drawn from the low temperature studies.

The third question will be investigated by a variety of conventional and special biochemical-biophysical techniques.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

N.B. The principle investigator would like to commend Miss Winter who has helped set this program up for her energetic, intelligent and responsible attitude. To our mixed, happiness and regret, Miss Winter is leaving the P.H.S. in January to get married.

Prepared by Bernard L. Strehler
October 28, 1957

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

1. a. Title: Studies on the differential fluorescent. Labelling of pituitary cells and granules obtained from them.

   b. Principal Investigator: Bernard L. Strehler (15% of time)

   c. Progress During Past Twelve Months: This project was begun about June 1957.

   d. Direction of Current Research: It is known that granules isolated from homogenates of the pituitary gland retain most of the hormonal activity of that gland. In an effort to ascertain whether the three sizes of granules observed in electron micrographs of pituitary homogenates (1) contain different hormonal activities and (2) originate in different cells we have been attempting to stain sections and granules differentially with various fluorescent dyes. (Fluorescence is a more sensitive indicator than is absorption spectroscopy.)

   A fluorescence microscope has been constructed for use in this and other preliminary work on the fluorescence of age pigments. Thus far, a systematic investigation of the use of about 20 different fluorescent stains at 8 different pH's plus numerous mixtures of these stains have afforded satisfactorily stained basophilic, neutrophilic and acidophilic cells and isolated granules.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals, Dr. Donald Mark, and Miss Anne Nesteruk
   (about 70% of the work on this project is being done by Dr. Mark and his assistant)

4. Publications and Awards: None

Prepared by Bernard L. Strehler
October 28, 1957

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

1. a. Title: Studies on the absorption and emission spectra of "age" pigments.

   b. Principal Investigator: Bernard L. Strehler (25% of time)

      Technical Assistance: Susanne Herman (30% of time)

   c. Progress During Past Twelve Months: This is a new project, begun about May 1957.

   d. Direction of Current Research: In collaboration with Dr. D. Mark of the Pathology Department, Baltimore City Hospitals, sections of heart, liver, kidney, brain and adrenals were prepared and examined in a microspectrophotometer constructed here with the assistance of Mr. Marvin Yiengst. The light source, monochromator and photodetector are adapted from the Aminco-Bowman Spectrophotofluorometer. Preliminary results indicate absorption maxima at about 300 mm. for some of these pigments and continually increasing absorption for others. In addition the fluorescence emission spectra of these pigments are being studied, photographically initially, later with more precision if the proper methodology can be derived.
   Goals and aims: To define some of the physical properties of compounds accumulating in aged animals.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals, Dr. Donald Mark, and Miss Anna Nesteruk

4. Publications and Awards: None

Prepared by Bernard L. Strehler
October 28, 1957

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

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: John Goffinet (Summer employment)
Theresa Caryk

Objectives: This project is designed to measure age changes in the morphology, chemistry, and function of separable protoplasmic particles. The working hypothec 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. Three main studies are proposed.

1. An age comparison of mean DNA content per nucleus in rat liver. This is designed to determine whether overall polyploidy actually increases with age as suggested by morphological observations. Nuclei isolated in dilute citric acid will be counted in haemocytometer chambers and DNA contents of these suspensions estimated by the Stumpf method.

In addition, hepatic and stromal nuclei will be separated by density gradient sedimentation in a small series of animals. The DNA content per nucleus of these two types will be determined.

2. An age comparison of oxidative phosphorylation in rat liver mitochondria. Mitochondria will be isolated in 0.25 m. sucrose by the Schneider method. Oxidative phosphorylation with a succinate system will be estimated by the usual manometric technique and results referred to mitochondrial nitrogen. Where possible mitochondrial counts and succinoxidase activity of mitochondria and whole homogenate will be used as additional reference standards and as estimates of mitochondrial recovery.

3. Ribonucleic acid (RNA) distribution study. Some evidence for an age derangement of protein synthetic apparatus was previously obtained (Barrows, unpublished). These were (a) low plasma, often low liver concentration of cholinesterase. (b) Increased liver concentration of RNA. To obtain further pertinent information it si proposed to determine the RNA distribution among three cell fractions: (1) combined nuclei and mitochondria (2) microsomes (3) supernate (not sedimented at 90,000 x g. for 70 minutes). RNA content of these three fractions and whole homogenate will be estimated by the orcinal reaction.

c. Progress During Past Twelve Months: Nuclear studies: Much of this was reported previousl . Since the last report the sucrose density gradient has been measured by means of bromobenzene-xylene droplets. This proved to be slightly sigmoid, i.e., steepest near the top and bottom of the tube. Size comparisons of liver nuclei (to the nearest 0.5) in sections and whole homogenate nuclei "stromal" the large "hepatic" due to temperature control difficulties, we still require runs on 4-5 more rats before this study can be considered complete.

Mitochondrial studies: The rat series in progress at last report has been completed. The previously suggested age decrement in intensity of oxidative phosphorylation (significant only to the 5% level) was not confirmed in this series. It is possible that the age decrement in this function described by Weinback and Corbus is confined to the growth phase and we are now investigating this possibility. Results with kidney suggest that the previously described age decrement in succinoxidase actively results from reduced numbers of mitochondria per unit wet weight rather than reduced activity per mitochondrian.

RNA study: This has been completed with respect to cell fractionation (n = approximately 35)and includes male rats young and old, of three nutritional states: 1. control 2. protein depletion 2. repletion. RNA analyses on 10 of these rats remains to be done so we cannot report results definitively. So, far, distribution changes with age and with nutritional status are not striking. RNA recoveries average 95% with about 55% recoverable in the microsome fraction. This agrees well with results in the literature.

d. Direction of Current Research: Density gradient fractionation of liver nuclei from 4-5 more rats will be performed with the aid of a newly acquired refrigerator.

Oxidative phosphorylation in liver mitochondria from young growing rats (aged 5 months) will be compared to that of 12 month old animals, with about 20 in each group.

Analyses on remaining fractions from 10 rats of the RNA distribution study will be completed.

More tentative goals include greater extension of techniques to other times such as brain and kidney as well as further applications of continuous density gradients. The clean separation of microsomes from mitochondria, fractionation of cell types in whole cell suspensions or isolation of "age" pigments are worthy aims.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Joseph A. Falson
October 28, 1957

Serial No.: NM-2[illegible]

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

1. a. Title: Studies on changes in localization and/or concentration of molecular populations during aging.

b. Principal Investigator: Bernard L. Strehler (5% of time)

Other Investigator: Marvin J. Yiengst

Technical Assistance: Susanne Herman (50% of time)
Patricia Winter (10% of time)

c. Progress During Past Twelve Months: This is a new project begun about March 1957.

d. Direction of Current Research: This research consists of a variety of approaches to problems of concentration, location and assay of important structural, catalytic or intermediary metabolic substances in living systems of various ages. Considerable time has been spent in setting up equipment and facilities for various analytical techniques including: pH gradient electrophoresis, paper chromatography of fluorescent labeled amino acids, continuous flow electrophoresis, ion exchange chromatography, bioluminescent assay of ATP and related compounds, agar block diffusion immunological techniques and the Coons technique for localization of fluorescent antibodies, etc.

Specifically it has been found possible to separate on two dimensional chromatograms mixtures of fluorescent labeled amino acids. Differences between young and old rats and between rats of the same age have been noted in the pattern of labeled spots but not all of the spots have yet been identified.

In collaboration with Dr. James Ebert of the Carnegie Institute of Washington, chickens have been injected with soluble muscle proteins from young and old rats. Work is in progress to determine the localization of soluble antigens in tissue cells and to determine the number, variability and distribution of antigens as a function of age and other variables.

2. Patients Days: None

3. Collaborators: Baltimore City Hospitals and Dr. James Ebert of the Carnegie Institute of Washington.

4. Publications and Awards: None

Prepared by Bernard L. Strehler
October 28, 1957

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

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. Currently two hypotheses are 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 then measurement with recognition techniques.

Hypothesis i. derives from the work of Welford and others, and is motivated by the ambiquity of experiments with the aged using the negative transfer paradigm.

Hypothesis ii. comes from work by Glick and the observation that recall is a more difficult task than recognition for any age group, and that there is a greater memory involvement in recall than recognition functions. Hypothesis ii has important implications for two other problems. Firstly, vocabulary has been used as a control for intellectual differences in cross-sectional aging studies on the assumption that verbal abilities as represented by vocabulary measures do not deteriorate with age. This study should indicate clearly whether recall, recognition, or either should be used as the controlling measure. (This aspect of this study is a part of project G-3 which is being dropped as a separate project.) Secondly, it is possible that differences between recall and recognition vocabulary are valid indicators of deterioration, and that these differences are in turn predictably related to the deterioration of other intellectual functions.

Method: Hypothesis i: The amount of interference as measured by anchoring effect on judgments of the sizes of squares will be observed for subjects of different ages. A tenable hypothesis would predict greater anchoring effect for older subjects. Subjects will learn to discriminate between five different sized squares. Then five larger squares will be introduced for discrimination. The first five squares will then be judged again. Only subjects who can learn to discriminate the original set of squares within a given number of trials will be retained. A control group will not receive the interpolated set of squares. This will show whether the interpolated set is actually interfering with the memory of the first set, or whether such deterioration would have occurred anyway. Effect is measured by the number of trials necessary to return to the level of

discrimination established with the first set of judgments.
Hypothesis ii: Young and old subjects will be matched on the basis of recognition scores obtained on a vocabulary test already developed. The difference in recall vocabulary scores, from another test already developed, will then be related to age.
Date of commencement: Early 1957.

b. Principal Investigators: George J. Suci (1/2 time)
Melvin D. Davidoff (1/3 time)

Technical Assistance: Dora Goldblatt (1/3 time)
John Braun (full time for summer only)

c. Progress During Past Twelve Months:
i. We have run 70 subjects living in the Infirmary of the Baltimore City Hospitals. Only 21 of these subjects met the criteria satisfactorily. With these subjects, whose ages range between 50 and 85, the results are in the predicted direction
ii. We have obtained recall and recognition scores from a large sample of enlisted airmen. This sample comes from a population restricted by the experimenter to include only high school graduates. Test material in proper format has been prepared and is ready to administer to older subjects. Golden Age Club members in Baltimore have been approached, but preliminary investigation has shown these subjects to be of rather low educational status. Other institutions for the aged are now being approached. A major problem will be to find a sizeable number of older subjects with high school education.

d. Direction of Current Research:
i. An attempt is being made to find more subjects, especially younger, uneducated people. If results are positive in the final analysis, we will consider further research on better defining the parameters of similarity between a set of stimuli and the interpolated material which interfered with the memory of this set of stimuli. In other words, we would like to be able to predict when interference with short-span memory will be greatest for the elderly.
ii. Data collection from older groups is now beginning. Our difficulty in obtaining proper subjects may cause this to take some time. When the data are analyzed we shall know whether to conclude the study or go on to studies of the ramifications of the results.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals, Golden Age Clubs of Baltimore and the U.S. Civil Service Commission was of assistance in obtaining data from the young group in the recognition-recall study.

4. Publications and Awards:

1. Reappointed "professorial lecturer" at American University in Washington, D.C. (not teaching at present time).

Dr. Davidoff will be devoting time during the year on preparing for publication material developed in studies carried on prior to his employment by N.I.H. These have to do with basic issues such as methodology in the determination of test reliabilities and test scoring. Alterations in a basic study of verbal abilities may also be made for publication if time allows.

Prepared by Melvin D. Davidoff
October 28, 1957

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

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 or no 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, and possible ramifications in central phenomena as well as those of deficit.

Working Hypotheses

i. 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.

ii. 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.

iii. 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 effect 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 effect 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 fall in this category of low CMS. The lowest level of CMS on this continuum would be observed in deep sleep.

iv. The activity level of the BSRS can be observed and recorded in intact human subjects by recording (electrically) the ampli-

of myotatic (stretch) reflexes. Presently available neurophysiological evidence strongly supports use of this method.
v. 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

i. 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. 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 both groups (aged and non-aged) 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.
ii. 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.
iii. Long-range plans include studies which will be concerned with the experimental manipulation of CMS in the aged through the use of drugs. We are also interested in studying the effects of irrelevant or distracting stimuli on CMS (attention) in a "continuous-vigil" type of task.

b. Principal Investigators: Walter W. Surwillo
Melvin D. Davidoff (1/3 time)

Technical Assistance: Raymond Flath*(1/5 time)

*Technical Assistance supported on National Heart Institute budge

c. Progress During Past Twelve Months: This project was initiated on July 28, 1957. Since that date all of the investigators' time has been applied to the procurement and construction of apparatus and the arrangement of facilities for the experiments. We expect to begin the first study by January 1958.

d. Direction of Current Research: See description of project above.

2. Patient Days: None

3. Collaborators: Baltimore City Hospitals

4. Publications and Awards: None

Prepared by Melvin D. Davidoff
October 28, 1957

GERONTOLOGY BRANCH

Project Report

January 1957 - December 1957

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. will be tested as follows: Stimulus material consisting of verbal messages of known difficulty as measured by the Flesch and possibly other methods will be read by subjects of varying age. The material will be encoded (repeated in the subjects' own words) onto recording tape. Errors of various kinds will be defined and measured. Techniques will be developed for measuring time consumed by pauses (time between words) and by verbalizations (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 (1/2 time)
Melvin D. Davidoff (1/3 time)

Technical Assistance: Carley Magee

c. Progress During Past Twelve Months:

i. Tape recordings of the free speech of some elderly subjects have been made and used in preliminary attempts to perfect techniques for recording time. It has been decided that more mechanical means of time recording are necessary and equipment for this purpose has begun to be investigated. One set of stimulus material has been worked up and discarded after preliminary test and another set is in the process of being developed now.

ii. Fifteen semantic differential type adjective scales and five concepts have been selected and prepared for administration. These will serve as a pilot study using Golden Age members as subjects to find whether or not elderly subjects are willing and able to do this kind of task. One Golden Age group has completed the task; nine other groups have been contacted and have agreed to complete the forms.

d. Direction of Current Research:

i. If errors and time of encoding do increase as a function of age the possibility exists that the aged person is a less efficient information handling machine. This would mean he is less efficient as a communicator as well. It is possible that frequency of error, type of error, and time increase, are functions of parameters of the stimulus material which is being encoded (e.g., its difficulty, its content, its structure, etc.). If this is so, it would theoretically be possible to increase communication efficiency by modifying stimulus material. Research along such lines would be planned.

ii. If the pilot study indicates that older people are able to perform on the semantic differential, then a full scale study with a more representative population of both stimulii and people will be initiated. One dimension that is measured by the semantic differential is attitude. It is hoped that a better knowledge of the attitudes of the elderly toward significant concepts, and the differences between these and the attitudes of younger people, will enable us to better understand (and therefore predict and explain) conflict situations involving young and old.

2. Patient Days: Not applicable

3. Collaborators: Baltimore City Hospitals, Golden Age Clubs of Baltimore, and probably certain local institutions for the aged.

4. Publications and Awards: A book involving the origin, development and ramifications of the "semantic differential" referred to above

has just been published. Osgood, C. E., Suci, G. J., and Tannenbaum, P. The Measurement of Meaning. University of Illinois Press, Urbana, 1957, 342 pp.

Prepared by Melvin D. Davidoff
October 28, 1957