ANALYSIS OF PROGRAM ACTIVITIES
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Summary of Intramural Research Activities

Laboratory of Chemistry of Natural Products

The research activities of this Laboratory are primarily directed to investigations of naturally-occurring substances which are significant because of their physiological action, their chemical structure, or their method of biosynthesis or biodegradation. These studies are characterized by highly exacting requirements covering a relatively large area of work. In almost every instance, the starting point for these projects requires extensive and careful isolation work, and usually a relatively small amount of material is obtained for study. Chemical studies of structure require much laboratory work and strong supporting facilities (often amounting to research level activities) in modern instrumental work. Following this stage, the work may turn to synthetic channels for purposes of structure proof, or to obtain materials for physiological studies. Alternatively, biological studies involving enzymatic transformations may be carried out on isolated or synthesized materials. During the past year experience in cellular fractionation techniques has permitted the carrying out of new and important studies on biological materials. Isolation studies on brain tissue have been in progress for several months, and this work, together with the enzymatic studies, constitute the chief new areas of activity of the Laboratory.

Three projects of the Laboratory deserve special mention. The hypertension project is aimed at finding new substances which affect the circulatory system, and emphasis is placed on the study of compounds which lower blood pressure. One of the chief results to be expected from this work is a greatly increased knowledge of the physiological mechanisms by which blood pressure may be controlled. The development of essential hypertension is clearly due to a failure of one or more of these mechanisms, but we do not yet know the site of action or nature of the failure which results in human hypertension. If new compounds can be found which have a specific hypotensive action, the chances of finding new drugs and finding new physiologic mechanisms involved in the control of blood pressure are greatly increased.

Four developments in this Laboratory during the year may be cited in this connection. A new compound of unknown structure (to

which the name panamine was given) was isolated from seeds of Ormosia panamensis, a tree native to Central America. By a simple functional group change, a potent hypotensive agent was obtained. The precise mode of action of this compound is still under study, but in dogs the effect is apparently exerted through a combination of central and peripheral action. The structure of the substance is not known, but present chemical evidence indicates a wholly new kind of structure not previously associated with physiologic action.

Clinical study of andromedotoxin, a potent hypotensive agent isolated from leaves of Rhododendron maximum, indicated that this substance was uniformly effective in humans, but that it had the same disadvantage as protoveratrine in intravenous work (nausea resulted from a slightly higher than therapeutic level), and it was not well absorbed on oral administration. With this result, work on related plants and on the structure of the compound was continued, but at a relatively low level. A procedure was developed for detecting andromedotoxin and several related (inactive) compounds in plants, and these techniques were also used in structural studies. One of the degradation products, prepared in a pure state, was found to have a marked hypotensive action. The pattern of action was different from that of andromedotoxin itself. For most known hypotensive agents, structural changes by degradation generally result in complete loss of activity and this unexpected effect makes clear the fact that we have relatively little knowledge of chemical structures which have a specific action on the controlling mechanisms of the circulatory system. Further research is the only means of learning more about these effects.

Studies on alkaloids of the Amaryllidaceae were continued, and the chemical work on the structure of these compounds is now approaching a successful conclusion. Several of these materials showed hypotensive action, but no particularly significant compounds were found, and extensive synthetic work is not planned for this field.

Near the close of the year several new observations were made on plant materials from Costa Rica and Puerto Rico. Indications of hypotensive action will be followed as rapidly as possible by isolation studies. In one instance a sedative action, somewhat resembling that produced by chlorpromazine (used in the treatment of mental illness) was found. This is an important development which requires further work. An active fraction has been isolated and is currently undergoing pharmacological evaluation.

These studies are organized to find, to evaluate, and to study the structure of new naturally-occurring compounds which affect the circulatory system, and it is clear that many such compounds remain to be found. At the same time, it is necessary to recognize that new and unusual products of plant or animal origin are the result of normal metabolic reactions, and that possibly some of these, or close relatives, are involved in human metabolism as well. This aspect of plant chemistry is often neglected, but it is responsible for a series of observations which led during the year to the initiation of new studies of considerable significance in human biochemistry in general, and in the biochemistry of the brain in a specific way. The origin of this work was the isolation of a series of indole alkaloids from seeds of Piptadenia peregrina (used in South America for the preparation of a hallucinogenic snuff). Two amine oxides were included in this group. The function of amine oxides in metabolic processes is not known, although they occur naturally and they are formed readily by enzymatic oxidation of amines (this has been demonstrated in this Laboratory). Careful chemical studies now show that it is possible that these amine oxides are key intermediates in the process by which N-methylation occurs in the body from a one-carbon fragment, and in the reverse process by which N-dealkylation occurs. If this is the case, it will constitute a major advance in our knowledge of fundamental biochemical mechanisms. An answer to this problem has been sought for many years and in many different ways, and it is hoped that the present work will provide the necessary key. This problem will be studied intensively during next year on both the chemical and enzymatic level.

A third broad project of considerable significance arose from the same initial observations on Piptadenia seeds. On the assumption that closely related indole bases would be found in the body, and that they would influence brain biochemical processes, a detailed study of brain tissue was undertaken. Our knowledge of biochemical processes in the brain is limited by the fact that we know very little about the nature of the substances which are present at hormonal levels, yet these materials have a profound influence on the brain. Studies in the Laboratory of Chemical Pharmacology (NHI) have demonstrated the necessity of knowing more about the composition of the central nervous system with regard to these substances, but isolation problems in this field involve much difficult and time-consuming work. The present study has resulted in the discovery of four new compounds of unknown structure in brain tissue. The compound which is present in greatest amount is evidently a tryptophan metabolite. It is a

neutral compound which is associated with brain lipids, and it may be quite important in the functioning of brain processes. Since this project is still in its early stages, it is not possible to evaluate the significance of this result, other than to say that a new avenue of research has been opened and the results may be quite important.

It may be of interest to compare the projects of this Laboratory with similar projects now under way in other research centers. Work on Ormosia compounds has been attempted in several other laboratories without marked success and without finding hypotensive action, but the chemical problem involved here is not likely to be solved quickly under any condition. The chemistry of andromedotoxin is currently under intensive study in Japan (Takemoto, Osaka) but their work has not yet been published. The work on Piptadenia compounds was initiated in this Laboratory, but the principal compounds involved in this study are now under rather wide investigation along chemical, biochemical and physiological lines in many laboratories. One of these compounds, bufotenine, is now supplied by the Upjohn Company for biological experimentation, and it is expected that a second one will also be available for this purpose. This Laboratory has cooperated with NIMH research units and with the Laboratory of Chemical Pharmacology, NHI, on this project. The new compounds found in brain tissue represent an advance which is not yet under study in other laboratories because of lack of material, but extensive further work in this field may be anticipated as soon as the first stages of work are completed. Chemical and enzymatic research studies on indoles are under way in most of the major medical research centers in this country and abroad, and this Laboratory is actively contributing to this field of work. Amine oxide studies are currently being pursued in a few University laboratories. The new results obtained in this Laboratory are now in process of publication and may be expected to influence the development of work in this field. Amaryllidaceae alkaloids are under study in England, Japan, Canada and Germany, with some additional work in South Africa. The principal work in this field in this country is in this Laboratory, and it is expected that this work will be brought to completion next year. Work in the general field of alkaloid chemistry has been primarily carried out in the past in Switzerland, England and Germany.

It is increasingly clear that chemical studies on biologically significant reactions should be accompanied by work at both the chemical and enzymatic level. This requires the cooperation and advice of biochemical groups, and points to the advantage of maintaining close contact between fundamental research groups

in adjacent fields. The rapid development of problems such as those involved in amine oxide work and Piptadenia compounds reflects a continuing development of the Laboratory along lines of combined organic and biochemical work.

Laboratory of Cellular Physiology and Metabolism - Cellular Physiology Section

During the year this section continued its investigations of basic biochemical phenomena at the subcellular and molecular level. The projects summarized below reflect the broad interests in the fields of protein, carbohydrate and lipid metabolism which the section maintains.

The Laboratory is actively engaged in a study of the enzyme lipoprotein lipase. Special emphasis has been placed on obtaining a relatively pure enzyme and on determining whether lipoprotein lipase, prepared from tissues of normal animals, contains protein-bound heparin. Adipose tissue has been found to be a rich source of the enzyme and a procedure has been developed for obtaining it from chicken fat in a more pure state than any previous preparation. Preliminary experiments using the bacterial "heparinase" described below as an analytical tool indicate that lipoprotein lipase may, indeed, contain heparin. Incubation of the lipase with the heparinase results in 60 per cent inactivation of the lipase. The nature of this inactivation is being studied. Another phase of this problem which is of current interest is the nature of the process by which the incubation of coconut oil with alpha-lipoproteins converts the oil into an available substrate for lipoprotein lipase. This appears to be a highly specific reaction in which the oil selectively adsorbs small amounts of protein, phospholipid and cholesterol. This system may be a useful model for the study of chylomicron formation. Both of these problems will be continued in the current year. This system is the only enzymatic mechanism known for the degradation of lipoproteins, substances whose abnormal metabolism has been associated with atherosclerosis.

A problem has been initiated concerned with the structure and metabolism of heparin. This compound, both because of its anticoagulant properties and its ability to induce the appearance of lipoprotein lipase in the circulation, has been the center of an immense volume of research yet its chemical structure and the mechanisms of its biosynthesis and degradation are unknown. The current studies have resulted in the isolation from soil of a Flavobacterium which is able to utilize heparin as its sole source of carbon. The

discovery of this microorganism provides a powerful tool for the study of heparin. Conditions have been developed for growing the bacteria on a large scale on a non-heparin medium and then adapting the culture to heparin utilization. Aqueous extracts of the acetone-dried cells have been obtained which are able to catalyze an extensive hydrolysis of heparin into relatively small fragments. It is known that the extract contains at least two enzymes which act on heparin, an alcohol sulfatase and a glycosidase. The products of this enzymatic hydrolysis of heparin are currently being isolated in pure form preparatory to their identification. When these degradative products are characterized it should be possible to picture the structure of the original heparin. The hydrolysis products will also be assayed for any biological activity which they might possess.

The study of the enzymatic degradation of cholesterol has been continued with major emphasis on the isolation and characterization of an unidentified co-factor(s) shown to be required for the degradation of the side chain to CO_2 by liver mitochondria. A reproducible method for the isolation of this co-factor in a relatively pure state from mouse liver has been developed which involved chromatography on charcoal, paper and ion-exchange resins. Microchemical analysis of this material has revealed that it contains organic phosphate, a reducing group, an alpha-amino nitrogen and a base in equimolar amounts. In addition to the continued characterization of this compound, it is hoped to isolate by enrichment cultures microorganisms which are able to degrade tocopherol and vitamin K, compounds with structures similar to cholesterol but of much greater solubility. The inability to degrade cholesterol may be involved in various pathologic conditions. An elucidation of the mechanism and required co-factors of the metabolism of this sterol is, therefore, of clinical as well as biochemical importance.

One of the major unanswered questions in intermediary metabolism is the mechanism of the conversion of the energy released during the flow of electrons along the terminal electron transport system of aerobic cells into high energy phosphate bonds. Heretofore the problem has been studied in intact mitochondria, an extremely complex system in which to define the individual steps of such a subtle process. Attempts have been made during the past year, with only limited success, to obtain soluble preparations from bacteria in which isolated parts of the overall system could be studied with greater refinement. It was demonstrated that extracts of Clostridium kluyveri catalyze the reduction of crotonyl-CoA to butyryl-CoA with reduced diphosphopyridine nucleotide serving as the electron donor. This was expected to be a useful

model system for the study of oxidative phosphorylation at the pyridine nucleotide-flavoprotein level. Both this system and a highly purified enzyme, pyruvic acid oxidase, from L. delbrueckii were investigated but it was not possible to obtain oxidative phosphorylation with either preparation. It is planned to employ some recently described procedures for the disintegration of mitochondria and bacteria to prepare soluble systems which still retain the ability to catalyze oxidative phosphorylation.

Studies on the enzymatic synthesis and utilization of energy-rich compounds have been continued. A new strain of Clostridium propionicum has been isolated by enrichment cultures. Enzyme preparations from this organism have been shown to catalyze the oxidation of propionate via acyl-CoA derivatives which include two thiol ester compounds now described for the first time: acrylyl-CoA and beta alanyl-CoA. The latter compound represents the only known amino acid-CoA derivative, a type of compound which has often been postulated to be an intermediate in protein biosynthesis.

Extensive investigations are being carried out on the intermediary metabolism of several interrelated amino acids – glycine, serine, lysine and proline – and one-carbon compounds. Co-factor requirements have been established for the reductive degradation of glycine and proline by partially purified enzymes of Clostridium HF. This study was greatly facilitated by the discovery that lipoic acid – an ubiquitous co-factor – can serve as the electron donor. An unidentified intermediate has been found to accumulate during the reduction of glycine. The conversion of serine to glycine is catalyzed by similar cell-free extracts. A number of folic acid derivatives which can serve as co-factors for this reaction have been isolated from boiled extracts of another bacterium Clostridium cylindrosporum. These have been partially characterized: 1) they are polyglutamates which contain 3 or 6 glutamate residues; 2) they contain varying amounts of several amino acids which are involved in serine metabolism; 3) they contain pentose and phosphate. It has been found possible to synthesize these derivatives enzymatically from teropterin (diglutamyl folic acid), inorganic phosphate, and serine. Upon the further addition of Mn ions, B_6 and DPN, glycine is formed from the serine. All of these studies will be continued with special emphasis on the enzymatic synthesis of P^{32}-labeled pteridine coenzymes and the details of the enzymatic reduction of proline and glycine.

The study of the structure of the enzyme ribonuclease has been continued with the aim ultimately to correlate specific structural features and amino acid sequences with enzymatic activity.

Information has been gathered with respect to the general amino acid sequence of the molecule, the sequences around the disulfide linkages which form bridges between several parts of the single chain, and those alterations in the molecular structure which destroy the enzyme's activity as well as those which are permissible. These efforts have permitted an educated guess as to what might be the "active center" of the molecule and an attempt will be made in the next year to synthesize by organic chemical methods those parts which are thought to be involved in biological function.

Laboratory of Cellular Physiology and Metabolism - Metabolism Section

The main areas of interest in the section on Metabolism over the past year have been (1) lipid and lipoprotein metabolism in relation to atherosclerosis, (2) the etiology and treatment of nephrosis, (3) mechanisms of protein synthesis and protein degradation, (4) assay of serum transaminase as a diagnostic aid in coronary artery disease, (5) studies on the structure of muscle protein, (6) studies on the mechanism of enzymatic decarboxylation.

(1) Several approaches to the problem of atherosclerosis are being followed. Fundamentally the laboratory effort is directed at elucidating the physiology of lipid and lipid protein metabolism in the hope that this basic knowledge will lead to a better understanding of the diseased state. It has been shown that the two major classes of normal serum lipoprotein - the beta$_1$-lipoprotein and the alpha$_1$-lipoprotein - are physiologically and chemically distinct. Isotopic studies in rabbits have shown that C^{14} labeled beta$_1$-lipoprotein and alpha$_1$-lipoprotein are not interconvertible at any significant rate. Using the DNFB method of Sanger it has been shown that the N-terminal groups of these two classes of protein are distinctly different. The beta$_1$-lipoprotein shows an N-terminal glutamic acid while the alpha$_1$-lipoprotein shows an N-terminal aspartic acid. The half-lives of these lipoproteins in the serum were found to be between 3 and 5 days approximately. An interesting implication that can be drawn from this observation, provided it is assumed the triglyceride moiety has a similar half-life, is that the number of calories transported in the lipoprotein fraction must be rather small.

Studies on the metabolism of serum unesterified fatty acids suggest that these may actually be a very important transport mechanism for fat. The arterio-venous difference of unesterified

fatty acids across the heart can, in the fasting individual, account for practically all of the caloric consumption of the heart. When glucose is administered intravenously this AV difference disappears. Further studies are in progress to examine the hypothesis that possibly all or nearly all of fat transport to and from the depots occurs in the form of unesterified fatty acids rather than in the form of triglycerides.

It has been previously shown in this laboratory that phospholipid exchanges rapidly between lipoprotein fractions in vitro. It is now demonstrated that free cholesterol also exchanges rapidly. Ester cholesterol on the other hand appears to be much more stably bound and exchanges only very slowly.

The initial disposition of ingested fat can be understood only in terms of chylomicron metabolism. The use of isotopic methods should make it possible to clarify many problems that have been difficult to approach by classical methods. Studies of the fate of chylomicrons labeled with C^{14} fatty acids and with C^{14}-cholesterol have been undertaken. Labeled chylomicrons disappear from the circulation with remarkable rapidity. This disappearance of radioactivity from the chylomicron fraction is not paralleled by a concomitant increase in radioactivity of low-density lipoproteins or of alpha$_1$-lipoprotein. Therefore it must be concluded that there is a mechanism for removal of chylomicrons or at least portions of the chylomicrons independent of a conversion of these into normal lipoprotein fractions. During clearing of labeled chylomicrons from the circulation there is a sharp rise in the specific radioactivity of serum unesterified fatty acids, indicating a role of lipoprotein lipase in the normal clearing process in agreement with previous results. Following the injection of palmitic acid-labeled rat chylomicrons into recipient rats it was found that the radioactivity accumulated predominantly in the liver and in the spleen.

Thorough study of three siblings with idiopathic hyperlipemia has uncovered a new metabolic deficiency. These patients, unlike any others so far studied, failed to produce lipoprotein lipase after the injection of heparin. While this is evidently a rare form of hyperlipemia, it is gratifying to be able to take at least one category of hyperlipemia out of the "idiopathic" wastebasket.

A program directed at the exploration of inhibitors of cholesterol synthesis has been initiated. Since endogenous synthesis accounts for approximately two-thirds of the daily cholesterol turnover, an effective inhibitor of cholesterol synthesis might prove valuable in treatment of hypercholesterolemia. The first compounds

studied, alpha-phenylbutyric acid and beta-phenylvaleric acid, while they inhibit cholesterol synthesis from labeled acetate, have proved to be of no value at the clinical level. In a series of approximately 20 cases, 6 of whom were maintained on rigidly controlled liquid formula diets, no significant response was observed, even in the patients with marked hypercholesterolemia. Δ^4-cholestenone, shown by Tompkins, et al., to be an effective inhibitor of cholesterol synthesis when given to rats in single massive doses, has been found to lower serum cholesterol of rats by as much as 45% when fed at a level of 1% in the diet over a six-week period. At this dose level, however, the compound is toxic. The most remarkable effect was a dramatic adrenal hypertrophy which is tentatively ascribed to an inhibition of steroid synthesis, although this is not yet firmly established. At lower levels of cholestenone intake it has been possible to lower the serum cholesterol levels without manifest toxicity and without adrenal hypertrophy. Studies are continuing directed ultimately at clinical testing of this compound. With the cooperation of Abbott Laboratories, several analogues of the intermediates in cholesterol synthesis are being tested for possible value in hypercholesterolemia.

Studies on the degradation of cholesterol by mouse liver mitochondria have led to the isolation of at least 4 acidic steroid breakdown products of cholesterol. These compounds are not identical with the common bile acids. One has been tentatively identified as 25-hydroxycholesterol.

(2) Clinical studies on the treatment of nephrosis with hydrocortisone and with the newer steroid compounds such as meticorten and meticortelone establish beyond question the value of this form of therapy. Eight out of 18 patients treated have had complete remissions (including 2 adults) and 6 others have had partial remissions. While it is too early to be sanguine about the long-term benefits, the results are most encouraging.

The concept that the antigen responsible for the formation of nephrotoxic serum in experimental animals is limited to the kidney no longer appears to be tenable. Significant amounts of the nephrotoxic antigen have been demonstrated in lung, placenta, heart, intestine and other organs. The soluble protective factor obtained by tryptic digestion of kidney has been found also in lung, skeletal muscle and heart and has been purified to the point where only 0.1 mg. of protein is required per protective dose. With further work on the nature of this protective factor it may be possible to explore the use of such a material in the prevention of clinical nephrosis.

A series of studies has been initiated to establish the nature of the immune response which has thus far made it impossible to successfully transplant kidneys. Blocking of this immune response might make feasible human kidney transplantation which represents the only hope for many patients with advanced kidney disease.

(3) In vitro studies of protein degradation in liver and kidney slices strongly suggest that there is a true dynamic state of intracellular protein, a concept recently questioned by Monod. These studies further point to a mechanism of protein degradation other than simple catheptic hydrolysis.

Further studies on the insulin inactivating enzyme of liver establish the proteolytic nature of this process beyond a doubt. The enzyme has been further purified and the sites of bond splitting have been partially identified. Several dithiohydantoin compounds have been shown to be effective inhibitors of the system.

(4) In collaboration with researchers at George Washington University Hospital, it has been shown in a series of over 200 cases that the determination of serum transaminase is an extremely valuable diagnostic aid. The use of this assay in differential diagnosis of coronary artery disease has been thoroughly examined. In 18 cases coming to autopsy elevation of serum glutamic oxalacetic transaminase has correlated 100% with the occurrence of recent myocardial infarction. It is clear that in an appreciable number of cases where the EKG is not diagnostic it will be possible to make an objective diagnosis using serum glutamic oxalacetic transaminase levels as a guide.

A simplified clinical assay procedure for this enzyme has been developed and is already finding wide application.

(5) It has been shown that the phosphorus content of myosin can be accounted for by the presence of a ribonucleic acid impurity. This nucleic acid has been isolated and characterized ultracentrifugally. A new heat stable protein unique in the absence of aromatic amino acids has been separated from myosin.

(6) The enzymatic decarboxylation of amino acids in the presence of H_2O^{18} does not lead to the incorporation of O^{18} in the released CO_2. These findings impose limits on any proposed mechanism of decarboxylation, in particular, ruling out the formation of a hydrated intermediate or of an acyl-enzyme complex. Decarboxylation of malonic acid, which is a coenzyme A dependent reaction, has been shown to occur beta to the coenzyme A linkage.

Laboratory of Technical Development

The general purposes of this laboratory are the development of new instruments and methods which may lead to new investigative technics in basic and clinical cardiovascular research. This requires the following areas of activity: (1) Basic investigations in physical, biophysical, and biochemical principles; (2) the development of instruments and/or methods for specific purposes; (3) modifications on existing instruments to extend their applications; (4) collaboration with other NHI laboratories in developing instruments and methods for their researches; and (5) providing consultation with other intramural and extramural investigators concerning the best approach for instrumenting their various problems.

The manner in which the above-stated purposes has been met is portrayed by the following brief descriptions of our more important projects. Several projects are continued from the previous year, several are new and have been completed, and the rest are new and will continue further. The projects are as follows:

1. Developments in Freezing Point Apparatus

Following the earlier development of the vortex tube refrigerating apparatus, Dr. R. L. Bowman has progressed towards a micro method for freezing point determination based on the idea that a chamber can be reproducibly cycled over a temperature range against time. A test set up capable of performing the cycling and visualizing the crystals in the micro sample is being tested and its problems evaluated. The system behaves as predicted, but requires further refinement.

2. Development of Spectrophotofluorometry and its Application to Biological Measurements

This project is a continuing one by Dr. R. L. Bowman, in cooperation with the Laboratory of Chemical Pharmacology. Evaluation of instrument requirements and a survey of the scope of applicability of ultraviolet fluorescence assay methods is in progress. A complete list of the pure compound survey is included in the progress report of Dr. Duggan of the Laboratory of Chemical Pharmacology. Methods of handling and purifying reagents for fluorescence work are being studied. Evaluation of the method of converting fluorescence to phosphorescence and separating exciting from emission light have been tested. While the method works well, it fails as yet to provide the increased sensitivity desired. Purity of glass is an obstacle to be overcome.

3. Development of an Ultramicroanalytic Method for Sodium and Potassium Determination in Micropuncture Samples

Since the previous report, Dr. Bowman has continued testing methods of eliminating the blank emission or making it constant.

The results indicated that the originally observed low blanks could not be depended upon for the various sources of quartz used. High purity quartz tubing recently obtained is now under test. Following this evaluation, the determination methods for the metals in question will be continued, in addition to Ca and Mg.

4. Development of Prosthetic, Therapeutic or Experimental Devices

Under this title, Dr. Bowman has collaborated with the Laboratory of Hemodynamics in the design and development of (1) a number of blood vessel coaptation clamps for anastomosing the aorta without suturing, (2) silastic valves for insertion in the aorta. Quite successful valve action was obtained in dogs, in spite of the poor mechanical advantage of the leaflets; pressure was maintained and the material appeared quite compatible. A successful, inflatable cuff renal artery clamp was developed for the Laboratory of Kidney and Electrolyte Metabolism. A bubble column - artificial lung oxygenator was constructed and tested for the surgical group, to test certain extravagant claims for the system. The results of test indicated that the bubble column is not the answer to the mechanical oxygenator.

5. Catheter Tip Pressure Gage

A new type of cardiac catheter tip gage is being developed and tested by Mr. Frank Noble. A high frequency sound wave is introduced into one lumen of a double lumen catheter. The cross sectional area of the sound path is varied by a diaphragm attached to intracardiac end of the catheter. The sound returns through the second lumen. Pressure applied to the diaphragm modulates the sound waves in accordance with the instantaneous value of the pressure so that the return lumen supplies an amplitude modulated sound to the receiving apparatus. The receiver demodulates the wave, yielding an output current proportional to the pressures. The test results have been very gratifying. Diaphragm arrangements and materials are being investigated further. When perfected, dog experiments will be run. Gastro-intestinal investigators have exhibited interest in the gage.

6. Function Generator and Fourier Analyzer

The purpose of this project by Mr. Frank Noble was to develop an instrument for easily and quickly obtaining the Fourier analysis of any biological wave form and for checking this analysis by synthesis. The frequency spectrum thus obtained specifies the characteristics required of an instrument which is to be used to record the wave form. The phase relations allow the synthesis for checking the analysis. This apparatus has been completed and is in use both by us and on request from other investigators.

7. X-ray Image Amplification and Cine-radiography

This project, conducted by Mr. Noble, is to provide facilities for making high speed motion pictures of the fluoroscopic image of man and experimental animals. A rough mechanical mounting for the image amplifier and 16 mm. camera has been made and tests begun. Motion pictures of the human heart have been made at a rate of 80 frames per second, utilizing Tri-x film and ordinary fluoroscopic currents. Film grain has been objectionable and Eastman is providing special films for test purposes. These are now under test.

8. Direct Coupled Electrokymograph

This instrument was designed and constructed by Mr. Frank Noble, for the purpose of having response down to true zero frequency. This characteristic was desirable to prevent distortion and to record the true motions of the heart borders and great vessels. Calibration of the motions and densities now become possible. This instrument is in use and tested by Dr. Dodge and Dr. Boone. When tests are completed, a final convenient model will be made and reported upon.

9. Self-Balancing Potentiometer

Dr. John L. Stephenson has developed a potentiometer type circuit of high sensitivity and rapid response. The earlier system using a string galvanometer was abandoned since adequate damping could not be obtained. Other type galvanometers are now used in conjunction with feed-back loops. Tremendous stability and high sensitivity have now been attained. During 6 months use in the 5 mv. range, a drift of less than 1% is present. A second system having a higher frequency range has also been found to be exceptionally good. These are being written up for publication purposes.

10. Physics of Ultra Rapid Freezing of Water, Collodial Solutions and Protoplasm

Dr. John L. Stephenson has continued his investigations on (1) the basic physics of the rapid freezing process in water, colloidal solutions and protoplasm, (2) applying this information to the analysis of hydration phenomena in protoplasm, and (3) extending the range of application of freezing and drying as a method of fixation and preservation of biological materials. Theoretical analysis of crystallization and cooling has been extended and technics for recording cooling curves have been improved.

11. Drying of Tissues

This project by Dr. Stephenson is aimed at obtaining information concerning the nature of the solid liquid interface in protoplasm. Standardization of the vacuum sublimation apparatus with ice is under way. A discrepancy between computed and measured evaporation rates for water has appeared and is outside the limit of experimental error.

This is being carefully investigated before proceeding further. Also, theoretical work on gas flow problems to drying has been extended and a paper on the results is being prepared.

12. Electron Microscopy

The project previously described by Dr. Stephenson, that of development of technics for electron microscopy of frozen-dried material, which was carried out in collaboration with Dr. I. Gersh of the University of Chicago has been essentially completed and the results are in preparation for publication. A new aspect of this work concerns the application of emission microscopy to biological materials and investigations in this direction are underway. Presently, in emission microscopy, the material to be examined is coated with cesium, which readily emits electrons when heated. These electrons can be used to form an enlarged image on a fluorescent screen or film. At present this technic is limited to ferrous metallurgy, our investigations are to evaluate its applicability to biologic materials. Preliminary results indicate this may be in the realm of possibility.

13. Development of a Capillary Type Viscometer for Measurement of Non-Newtonian Fluids at Low Concentrations

The objective of this project by Dr. Murray Eden is to develop and test a capillary viscometer for studies in the kinetics of degradation of high molecular weight substances, such as DNA or hyaluronic acid. Considerations suggested that measurements at very low shear rates could be used. Tests on the initial model showed it to be too cumbersome to operate. A second model, utilizing a modified analytical balance was developed. This present model shows good promise and is being evaluated and tested.

14. Development of a Micro Glass Electrode

Dr. Murray Eden has been developing a glass electrode small enough to be imbedded in living organisms for a length of time and to function as a sensing element for the measurement of pH. Micro glass electrodes were fabricated from Corning 015 glass. The diameter of the tip varied from 2 to 5 microns. The resistance of these electrodes was in excess of 1000 megohms. The preparative technics will be improved to cut down on the number broken in handling. Investigations are under way on materials for coating the electrodes. The pH response of these high resistance electrodes will be studied utilizing a vibrating reed electrometer.

15. Development of a Probabilistic Model for Growth

Dr. Murray Eden has been developing a minimal information, stochastic, two-dimensional model of a growing organism and studying the properties of the model. The model is in a sense an analogy to biological growth and the results may give some insight into the minimal information content required for biological growth. The

sample of configurations of 1024 cells will be generated and studied utilizing the Institute for Advanced Study Computer at Princeton. An attempt will be made to relate the model to certain biological studies such as the Weiss tissue culture and Freeman on twinning. If a suitable organism, such as Alga, is found, attempts will be made to compare the algal growth to the model predictions.

Laboratory of Chemical Pharmacology

Role of Serotonin in Brain Function. Our studies now indicate rather strongly that serotonin is a chemical mediator, important in the function of the brain, and that changes in central nervous system function can be caused by changes in serotoin levels in brain.

Reserpine and other tranquillizing Rauwolfia alkaloids block the mechanism that allows certain cells, including those of brain, to maintain serotoin in a "bound" form. As a result serotonin is released from its "binding" sites and metabolized by the action of monoamine oxidase. Serotonin continues to be made but cannot be localized in cells and is now present in brain in a free state. Reserpine also releases serotonin in vitro (platelets). This is not a simple physical displacement but seems to involve a complicated biochemical reaction with one molecule of reserpine releasing hundreds of serotonin.

The reserpine effect on serotonin "binding" sites is irreversible. Thus, brain cells are unable to take up serotonin long after reserpine has disappeared from the brain. The pharmacologic actions of reserpine are temporally related to the serotonin change and not to reserpine concentration in brain and persist until new acceptor sites for serotonin are formed. It is probable, therefore, that reserpine action is mediated through the change in the state of serotonin.

Not only the tranquillizing action, but also the lowering of blood pressure, temperature, slowing of heart rate, miosis, ptosis, increase in intestinal movement, etc., are mediated through serotonin. Presumably, therefore, serotonin has a role as a chemical mediator in the central nervous system, and perhaps the gastrointestinal tract.

Marked increases in free brain serotonin can be induced by giving reserpine after first blocking monoamine oxidase by pretreatment with Marsilid, or by giving 5-hydroxytryptophan, the amino acid precursor of serotonin. The animals exhibit the typical symptoms (aberrant brain function) seen after giving the hallucinogenic agent LSD, which is thought to produce a functional decrease in serotonin by blocking its action. The reversal of serotonin action by its presence in excess in a free form is consistent with the concept of it being a neurohumoral agent. Pyridoxine deficiency, which lowers brain serotonin, and Marsilid, which increases brain serotonin, also produce marked central disturbances.

Although reserpine itself is present for only relatively brief periods, its effects are cumulative when given to man in small daily doses. Binding sites are progressively knocked out until finally little bound serotonin is present in platelets. These results explain the slow onset of action, the long duration of action after interruption of dosage, and the potential dangers of this type of drug. No change in bleeding time in patients or animals with serotonin depletion, indicating that contrary to popular belief it probably has no role in hemostasis.

Other data point to a role of serotonin as a neurohumoral agent. Thus, serotonin, its synthesizing enzyme and its destroying enzyme are all concentrated in the brainstem, especially the hypothalamus.

Chlorpromazine, a synthetic drug with many actions similar to those of reserpine, does not act directly through serotonin. How it fits in the serotonin picture is not as yet clear. It is metabolized to chlorpromazine sulfoxide, which also shows tranquillizing activity in dogs and cats but with minimal production of postural hypotension. The sulfoxide is being tested in man as a drug for the treatment of mental illnesses.

Patients with malignant carcinoid of intestinal origin, a tumor which produces hugh amounts of serotonin in the body, have been studied. In spite of high platelet serotonin, bleeding times are normal. The disease can be diagnosed on the basis of urinary excretion of 5-hydroxyindoleacetic acid. These studies have been useful in confirming, in man, some of the biochemical aspects of serotonin metabolism and in suggesting some additional physiologic and pharmacologic effects of serotonin, especially on the heart.

Detoxication of Drugs. Further studies have indicated even more strongly that enzymes that inactivate foreign compounds show unusual differences from those required in normal body economy.

Enzymes that change drugs oxidatively have been further studied. To the list of those which are in microsomes and require O_2 and TPNH can be added the one that oxidizes chlorpromazine to the sulfoxide. The mechanism of the various oxidative reactions has been studied. The oxidation of TPNH produces a peroxide-like material. It is possible that this material is a common intermediate for a series of nonspecific enzyme systems that split ethers, oxidize sidechains, dealkylate amines, hydroxylate rings, etc.

Evidence is accumulating that these microsomal enzymes serve no purpose other than a protective mechanism against foreign compounds. They are absent in a number of lower animals, some of which apparently excrete drugs unchanged.

These enzymes are probably a "primitive" type since nonenzymatic model systems have been found which hydroxylate rings, split ethers,

and dealkylate amines. These model systems have supplied us with unusual tools for studying the mechanism of enzymatic reactions.

Reductive enzymes are also under study. Reduction of nitro and azo compounds to amines involves mechanisms that are present mainly in the cytoplasm, but are also present in microsomes of liver and kidney. They operate with either TPNH or DPNH but are different enzyme systems. They are probably flavoproteins but differ from any known flavoprotein since they are activated by all three flavins, FMN, FAD and riboflavin. The addition of large excess FMN accelerates greatly the metabolism of nitro compounds, an observation of considerable potential importance to toxicology, since no way, as yet, is known that will increase biotransformation of drugs.

Mechanisms of Potentiation. Potentiators are of at least two types: Prolonging agents which inhibit metabolism of drugs and true potentiators (chlorpromazine and reserpine) which make sub-effective doses of drugs effective. A number of compounds (SKF 525, Eli Lilly 18947, Marsilid, etc.) of quite different structure block the metabolism of the same wide spectrum of drugs, including those undergoing oxidation, reduction, hydrolysis, and conjugation. These types of actions seem to have nothing in common, even as to localization in cells. The mechanism of this blocking effect remains a mystery.

Studies with Ascorbic Acid. Certain mammals, including man, cannot make ascorbic acid because they are unable to transform free glucuronic acid to ascorbic acid. The increased formation of ascorbic acid in certain mammals after administration of foreign compounds of unrelated structure and pharmacologic activity is not associated with their detoxication, since barbital which is excreted unchanged also produces the result. Guinea pigs, which do not synthesize ascorbic acid, show an increase in glucuronic acid formation. The physiological significance of this response of the body to foreign compounds is under investigation.

D-ascorbic acid when maintained at high levels in guinea pigs, can replace L-ascorbic acid as far as maintenance of weight and survival are concerned but does not prevent marked hemorrhages in knee joints. This suggests a poly-functional role of L-ascorbic -- one non-specific and the other specific.

Blood-Brain Barrier to Drugs. It is important to know the relationship between chemical structure, physicochemical properties, and the ability of drugs to pass the blood-brain barrier. Preliminary results indicate a barrier of a lipid nature, since the rate of passage into brain of many drugs parallels their fat solubility. Thus, the barrier shows different properties for drugs than it does for electrolytes and normally occurring organic substrates for which selective permeability exists.

Absorption of Drugs. Previous results have shown that the barrier between blood and stomach acts as a lipid membrane to organic compounds and accounts for the observation that organic acids, if

soluble in acid solution, can be absorbed directly from stomach. Preliminary results on intestinal absorption indicate that the barrier between blood and intestinal tract acts as lipid-type membrane for bases. For instance, very strong bases are absorbed with difficulty and weak bases with ease. However, the absorption of organic acids is more complicated since many of them are apparently absorbed in the anionic state.

Dry Screening. This program is conducted in collaboration with Dr. Terry's group and the group at Goldwater Memorial Hospital.

A muscle relaxant, Flexin, with long-lasting myaresin-like activity is metabolized to a compound which has similar pharmacologic activity.

Chlorpromazine sulfoxide, a metabolite of chlorpromazine found to have tranquillizing effects, is also sedative in man. It is too early to say whether it will be useful in treatment of mental illnesses.

Mephentamine sulfate acts as an antiarrhythmic drug probably by a mechanism different from that of quinidine and procaine amide, that is, by masking ectopic activity by increasing the sinus rate.

In looking for phenylbutazone analogues with anti-inflammatory activity, it has been shown that changes in the butyl sidechain reduce antirheumatic activity but usually increase uricosuric effect. Substitutions in the benzene ring retain antirheumatic activity. Unfortunately, a large series of compounds made by Geigy have proven to be too insoluble for absorption from the gastrointestinal tract. More soluble analogues are being made.

The active principle of Ormosia panamensis has profound hypotensive properties of obscure mechanism. It is being evaluated in man.

Autonomic Nervous System. After the production of experimental myocardial infarctions in dogs arrhythmias occur spontaneously for 3 to 4 days. For the next 10 days there is no spontaneous arrhythmia, but extremely small doses of epinephrine or norepinephrine cause arrhythmias. It is possible that a similar hypersensitivity to epinephrine causes the tachycardia often observed after occlusion in man.

A method has been developed to measure contractile force by using a strain gage sutured directly to the surface of the heart. This experimental setup may be used to screen digitalis-like drugs. Using this technique it has been shown that reflex production of vasomotor discharge by temporary occlusion of the common carotid arteries produces no change in cardiac contractile force but does produce a marked vasopressor response. It is concluded from this and other types of experiments that the central nervous pathways involved in control of the force of contraction of the heart and in the control of blood pressure are different.

Methodology. The spectrophotofluorometer has been further developed and applied. A large number of aromatic drugs and normally occurring hormones and vitamins can be assayed with the apparatus. There is little doubt, now, that it will drastically change certain aspects of analytical chemistry.

Laboratory of Cardiovascular Hemodynamics

Perhaps the largest part of this laboratory's efforts have been devoted to a study of the oxygen requirements of the myocardium and the determination of those components of heart activity which dominate its oxygen requirement. It has been found that stroke volume "costs" little and that the development of tension is "expensive". It develops therefore that the correlation between work, per se, and oxygen consumption is spurious. Of further interest is the fact that relationship between diastolic filling pressure (fiber length) and ventricular work is a selective one. That is, an increase in ventricular work caused by increasing aortic pressure is accompanied by a substantially smaller increase in filling pressure than a comparable work increase caused by increasing stroke volume. The combination of these two considerations constitutes a negation of the second part of Starling's Law of the Heart and introduces the possibility of a more definitive understanding of myocardial activity.

Techniques have been developed which make it possible to substitute a plastic valve for the aortic valve. The development of a new valve has been accomplished which, unlike previous valves, has an acceptably low traumatic effect on red blood cells. It is hoped that this development presages definitive valve replacement rather than approximate valve repair in human valvular disease of the heart.

By means of precise pressure and flow metering techniques it has been possible to initiate a quantitative study of the hemodynamics of valvular insufficiency, in this case mitral insufficiency. A number of interesting and original observations have been made among which is the surprisingly large volume of regurgitant flow that can be tolerated with only a minimal effect on left atrial pressure. These observations suggest that regurgitant volumes in this disease are many times in excess of what was previously suspected.

Fundamental studies of the pathologic physiology and treatment of cardiac tamponade have been completed. Two findings of significance were obtained. The first, of physiological importance, is that the myocardial fiber can be induced to contract more forcefully from a restricted diastolic fiber length by the use of sympathomimetic agents. The second, of possible clinical importance, is that the use of such agents may be of help as a holding maneuver in clinical tamponade.

These observations on tamponade have been, in turn, a part of a larger study on the circulatory derangement during high levels of

positive pressure breathing in which, we now believe, cardiac tamponade plays an important role. Further, the use of sympathomimetic agents decreases the distensibility of peripheral blood vessels (demonstrated in vitro) and substantially improves the circulation for significant periods of time by diminishing the displacement of blood from thorax to periphery.

A program of investigation has been carried out on the reactivity of isolated blood vessel strips under carefully controlled conditions. The effects of changing the chemical environment (with particular reference to potassium) and of a variety of pharmacological substances, including steroids and sympathomimetics, has been ascertained.

Members of this laboratory have collaborated with other laboratories on various clinical problems as, for example, problems in congenital heart disease and idiopathic postural hypotension (and the treatment thereof with pitressin).

Laboratory of Kidney and Electrolyte Metabolism

The work of this laboratory continues to be directed toward an increased understanding of the mechanisms involved in the transport of water and electrolytes across cell membranes and clarification of the ways in which these processes are integrated in the regulation, by the kidney, of the fluid and electrolyte content of the body. The derangements of renal regulation in heart failure and other disorders are studied both experimentally and clinically. Current investigations can be conveniently divided into three groups although these are not mutually exclusive and several projects extend into all three. These categories are: 1) studies concerned with the distribution and transport of fluid and electrolyte across cell membranes and secretory tissues; 2) investigations of the kidney mechanisms involved in the regulation of fluid and electrolyte balance; and 3) research in disorders of fluid and electrolyte metabolism in experimental heart failure and other disorders associated with abnormal accumulations of fluid in the body.

Studies aimed at elucidation of the mechanisms by which cells maintain their internal environment by the uptake of potassium and extrusion of sodium have continued, using red blood cells as the tissue most amenable to quantitative study. The importance of certain metabolic pathways has been demonstrated by the use of inhibitors of various enzymic reactions. A search among various phosphorylated compounds for one the potassium salt of which might have a particularly low activity coefficient and hence be a possible specific membrane carrier has so far proved fruitless.

The development of technics for labelling inulin with carbon-14 and measuring its concentration at very low levels has made it

possible to obtain more definitive estimates of the volumes of fluid inside and outside of cells and hence to evaluate more accurately the transport of substances across cell membranes in intact tissues. This technic has been applied to the study of the movement of chloride across the frog stomach and the results indicate that the turnover of chloride is far more rapid at the secretory than the nutrient surface. Preliminary experiments have been carried out aimed at the application of the radioactive inulin method to the measurement of the extracellular fluid space of individual tissues and of intact animals and to what may prove to be a greatly simplified procedure for the estimation of renal function.

Work has progressed toward the isolation and identification of a naturally-occurring steroid substance which exerts a digitalis-like effect on heart muscle. This material, present in normal blood and tissues, is found in highest concentration in adrenal medulla. It is closely associated with a lecithin-like material from which it has not yet been possible to separate it without loss of activity. Work to explore its physiologic significance has been undertaken. Meanwhile the interesting and possibly important preliminary observation has been made that the concentration of this cardiotonic substance (which also causes increased contraction of arterial walls) is uniformly increased in patients with "essential" hypertension but not in a limited number of individuals with other disturbances.

Work on the transport of water and electrolytes by the kidney has made significant progress. The technic of measurement of glomerular filtration rate has been greatly simplified and work thus accelerated by the demonstration that the more easily measured alkali-stable inulin has the same clearance as total inulin. The unique anatomical arrangement of the renal circulation in the chicken has made possible experiments in this species which indicate that the secretory transport of potassium is effected by a mechanism subject to saturation at high loads demonstrate more directly than has heretofore been possible, that the secretion of potassium and hydrogen ions are reciprocally related, and show unequivocally that mercurial diuretics specifically inhibit potassium secretion. An interesting, and as yet unexplained, specific effect of ammonium ion on the reabsorption of chloride has been found and is to be further explored.

Studies involving the reduction of glomerular filtration in one kidney by constriction of the renal artery on one side during water diuresis in the trained unanesthetized dog have served to clarify considerably the mechanism of urine dilution and the mode of action of antidiuretic hormone. It has been possible to show that every reduction in glomerular filtration is associated with a rise in urine concentration and that when the filtration rate is sufficiently reduced the urine becomes hypertonic despite complete absence of antidiuretic hormone. The results strongly support the view that urine is diluted by solute reabsorption, that the effect of antidiuretic hormone is on the permeability of the tubule to water and that the mechanism responsible for the production of hypertonic

urine is not dependent upon this hormone.

Complementing this work are studies on patients with pitressin-unresponsive diabetes insipidus and in normal subjects which indicate that the excess water excretion (free water clearance) in water diuresis is directly related to the rate of solute excretion. These results indicate that, even in the absence of antidiuretic hormone, water "freed" by solute reabsorption diffuses from tubule lumen to blood and at a rate proportional to the concentration gradient. These studies, along with the preceding, contribute importantly to an understanding of the impairment of water excretion in heart failure and other abnormal states.

Research on the collection and analysis of specimens from the lumen of individual kidney tubules has made slow progress. Techniques for the experimental manipulations have been improved and a few preliminary measurements of the electrical potential across the renal tubule have been made.

A method for the assay of diuretic agents involving the use of dogs with ascites due to inferior vena cava constriction has been evolved and promises to facilitate work in this field.

Previous studies indicating excessive secretion of salt-retaining adrenal steroids in dogs with experimental heart failure and in dogs with ascites due to constriction of the inferior vena cava have been confirmed and made more definitive by the isolation of increased amounts of aldosterone from the urine and from adrenal vein blood. It has been possible to show the accumulation of appreciable volumes of ascitic fluid in the absence of net positive sodium balance in dogs maintained on a salt-free intake and subjected to constriction of the inferior vena cava. This strongly supports a "backward failure" type of mechanism in this preparation.

Clinic of Gerontology

The Section on Gerontology has been concerned with age changes in the cardiovascular-renal system, metabolism and endocrinology, pulmonary physiology and the response to exercise.

There is a gradual decrement in pulmonary function with age; this appears as a reduction in vital capacity, total lung volume and maximum breathing capacity, with an increase in residual volume. The reduction in vital capacity is largely a reflection of the overall reduction in body size with age. The important age change is a gradual increase in residual volume at the expense of vital capacity. It has also been found that the reduction in maximum ventilatory volume in the older ages can be attributed to a reduced increment in the voluntary rate of ventilation and not to a reduction in the tidal volume attained. It is proposed to relate the laboratory measurements of

pulmonary function to clinical evaluations in older subjects with signs of pathology. It is also planned to extend the measurements to other dynamic indices of pulmonary physiology in order to specify more completely the factors associated with age changes.

Studies on age changes in thyroid function are in progress. Indices of thyroid function include measurements of basal metabolism, rate of uptake of I^{131} by the thyroid gland and levels of protein bound iodine in the plasma. An isotope laboratory has been set up and the methods for determining the rate of uptake by the thyroid gland have been standardized. Estimates of basal metabolism and total body water content in females have been made. In the group thus far tested, the basal oxygen uptake per liter of body water does not change with age. Thus, further studies of thyroid function must be made before the commonly assumed "metabolic slowing" with age can be accepted. It is proposed to investigate the response of the thyroid gland in older people to the administration of TSH. The responses to thyroid administration will also be investigated.

Carbohydrate metabolism, as evidenced by the rate of removal of intravenously administered glucose from the blood, is reduced in older subjects. Furthermore, the rate of removal of glucose is increased more in young than in old subjects when a standard dose of insulin is administered along with the glucose. Thus, age differences in the response to a metabolic stimulus have been demonstrated. Additional studies, utilizing other substrates, are planned.

Old subjects show a greater increase in ventilation following standard amounts of exercise than do young. The amount of oxygen required for the exercise does not change significantly with age, except at slow rates of work. When working very slowly, the old individual requires a greater amount of oxygen for a given amount of work than does the young. Thus, extremely low rates of work are of benefit to the older subject only because the total demand for oxygen is less, but the oxygen required per Kg.M. of worker is greater. Experiments to test the hypothesis that the reduced efficiency of the older subjects is due to impaired coordination are planned; if this is true, training programs could be developed that might be useful in older people. In addition, measurements of the efficiency of simple movements will be tested with instrumentation that has been designed in the Section.

An age difference in the response of the peripheral vascular system to vasodilitation has been found. In older subjects, limitations in peripheral vascular function may be encountered without the presence of clinically demonstrable disease. Using a unique method developed in our laboratory whereby the rate of propagation of a pressure wave experimentally induced in the intact artery is measured, age changes in the viscous and elastic properties of the arterial wall have been described. The changes in brachioradial behavior have been characterized as parameters in a descriptive equation. Differences have also been identified in the behavior of the brachioradial arteries of hypertensive and normotensive subjects

at comparable pressures and age, and of clinically "sclerotic" and "non-sclerotic" subjects of comparable ages. These differences provide a basis for interpreting the changes with age as indicating a reduction in the parameters denoting muscular participation as well as the development of the "sclerotic" pattern in this muscular artery. The calculation of cardiac output from formulae based on blood pressure curves is only approximately valid under conditions of rest. When sinusoidal force is applied to the body over the frequency range used in clinical ballistocardiography, the body does not respond as a unified mass, thus refuting one of the assumptions made in ballistocardiographic interpretations. Thus, age changes in the cardiovascular system have been demonstrated which are not detectable by the methods used for diagnosing disease states.

Studies on the age changes in enzyme activity of the heart, liver and kidney of the rat have been started. Preliminary results fail to show any striking age changes in the resting oxygen uptake of these tissues when appropriate corrections (DNA content or protein N content) are made for age losses in functioning tissue. Specific enzyme systems are under investigation. There is apparently a reduction in the succinoxidase activity in heart and kidney which is greater than the reduction in functioning tissue. The hypothesis that the reduction in enzyme activity preceeds the loss of cells is being investigated.

Thus, in addition to describing quantitatively some of the physiological changes that take place with aging in the human, the mechanism of these changes has been specified in some instances and a beginning has been made in studies at a more basic level, using animal tissues. It can be said that, although part of the impairments of older people can be ascribed to a gradual loss of functioning tissue, there apparently are functional changes that occur in the cells prior to their disappearance.

Clinic of General Medicine and Experimental Therapeutics

A. Study of Antihypertensive Drugs. Working in collaboration with the Laboratory of the Chemistry of Natural Products and the Laboratory of Chemical Pharmacology we have succeeded in setting up screening procedures which allow us to evaluate systematically the efficacy and toxicity of new drugs for use in hypertension. These studies have yielded, also, valuable basic information relative to the mechanism of action of certain of these drugs. The results of the studies of reserpine and its relation to serotonin and other vaso-active substances is an excellent example. The basic mechanisms responsible for a new clinical syndrome, malignant carcinoid, have been elucidated for the first time. This basic understanding has led to other important fields such as the normal metabolic pathway of tryptophan and the physiologic effects of varying quantities of serotonin in the body.

During the year laboratory methods have been set up for the measurement of catechol amines in the blood and urine of patients. This has led to the correct laboratory diagnosis of pheochromocytoma in several cases and it also gives us a laboratory tool which is of great potential value in the study of the mechanisms associated with other clinical conditions which are accompanied by arterial hypertension.

B. The Mechanism of Action of Aldosterone. The broad general field of the role of the adrenal in salt and water metabolism is being studied intensively. Special attention has been given to aldosterone since it is the most potent natural steroid related to the retention of salt and water in congestive heart failure and other disease states. Chemical and bioassay techniques have been devised and perfected for the detection and quantitation of this important steroid. With these tools the mechanism of action of aldosterone has been studied with the ultimate hope of learning why its secretion is excessive in patients with edema due to heart disease. Much of this pattern has already been determined and it is hoped that the entire mechanism may be elucidated in the near future. Such basic information would contribute considerably to the therapy and management of patients with heart disease.

C. Studies with Synthetic Adreno-cortical Steroid Analogs. Chemical and pharmacological studies have been conducted to elucidate the character and mode of action of a group of new synthetic steroids. It is the hope that these studies will help in finding new therapeutic tools and in gaining additional insight into the relationship between structure and function of the steroids. These substances have been studied in experimental animals and then in patients on a strict metabolic balance regimen. These studies have been applied to several series of new corticosteroid analogs and have already revealed several groups of these compounds which have important usefulness in the treatment of patients. Further studies are planned with these and other related substances in order to correlate further the chemical structure and function of such compounds.

D. Studies of Ventricular Dynamics. These studies have been performed in man with the primary objective of learning more about what factors affect ventricular function and thus cardiac efficiency. A group of patients with atrial fibrillation has been studied hemodynamically and has given important information relative to cardiac efficiency. For the first time a demonstration in man of the applicability of Starling's Law of the Heart has been provided. Further studies along this line are expected to add greatly to our understanding of the basic mechanisms affecting the functional efficiency of the heart.

E. Development of a New Flowmeter. Incidental to studies of certain flow characteristics of blood in animals, a new type of plastic flowmeter has been devised. The "orifice-type" meter is now being subjected to critical study. Evidence to date suggests that this may

constitute a major contribution to experimental medicine by providing a more satisfactory instrument than has been available in the past. The problem of a satisfactory measurement of flow in the vessels of living animals has always been a great obstacle in physiological studies. If this meter proves to be as good as it now appears it will provide a major advance in this field.

F. The Recording Characteristics of Various Pressure Measuring Systems. Investigators have been using various pressure measuring devices in medicine for decades. Recent engineering developments have allowed for more accurate measurements in recent years. However, there has been considerable difficulty in comparing and correlating large segments of these data because of the lack of information relative to the characteristics of the various systems. One of our investigators has completed a systematic evaluation of such systems under the most carefully controlled experimental conditions. The stability as well as the static accuracy and dynamic accuracy have been carefully determined. The results of this study are now available and make a significant contribution in this field of experimental medicine.

Clinic of Surgery

The clinical investigative projects for the past year have been generally concerned with patients with valvular heart disease and those with intracardiac shunts. The technique of left heart catheterization has been extensively applied in these studies. A correlative study of left atrial pressure pulses in patients with mitral valve disease has indicated that this is the most effective method of assessing the degree of mitral insufficiency accompanying mitral stenosis. In the absence of a valvular lesion it has been found that the left atrial filling pressure reflects the degree of left ventricular failure present.

Catheterization of the left ventricle has afforded an opportunity for the measurement of the gradient across the aortic valve in the presence of aortic stenosis. In the patients studied, it has been found that a systolic gradient of 100 to 250 mm. of mercury exists when the patient has symptoms. Gradients of 50 to 60 have been observed in early forms of the disease. Following successful aortic valvulotomy this gradient is reduced to 20 to 30 mm. of mercury. It is believed that the measurement of the aortic gradient furnishes a reliable method for the selection of patients for aortic valvulotomy and assessing objectively the effectiveness of the operation.

Left heart catheterization also furnishes a measurement of the diastolic gradient across the mitral valve. We are thus able to precisely determine the presence of mitral stenosis and draw conclusions as to its severity. Diastolic gradients of 15 to 35 mm. of mercury are generally seen in patients selected for operation.

Following successful commissurotomy the gradient is either completely abolished or greatly reduced. A certain number of patients have presented with the clinical picture of mitral stenosis but have been shown by this technique to have a normal mitral valve. These patients have primary myocardial disease.

Some twenty patients have been studied by a new technique of central aortic catheterization. The catheter is passed from the femoral artery to a position just above the aortic valve. Pressures are then recorded from this area and from a peripheral artery simultaneously. It has been found that the abnormal pulse contour associated with aortic stenosis is much more prominent in the central aorta than in a peripheral artery and minimal degrees of aortic stenosis may thus be detected. The differences between central and peripheral pulse pressure seem to reflect the severity of aortic insufficiency. Central aortic catheterization appears to be a useful tool in the selection of patients for aortic valve surgery.

Several other techniques have been extensively used in the study of patients with interatrial and interventricular septal defects. In addition to the usual right heart catheterization, the magnitude of shunts has been determined by measurement of differential nitrous oxide levels from the various heart chambers. This method is much more sensitive than the determination of oxygen differences and nine patients have been proved to have interatrial septal defects which would not have been detected by routine study. The size of interatrial septal defects has been measured by a special catheter with an inflatable balloon attached. The catheter is passed through the defect and inflated in the left atrium. By adjusting the size of the balloon until it will just pass through the defect an accurate indication of the size and location of the communication can be made. By selective angiocardiography in the left atrium, additional information concerning the relationships of the defect can be obtained.

Hypothermia has been used clinically in some thirty patients. The technique of atrio-caval infiltration devised in this laboratory has been found effective in the prevention of ventricular fibrillation during hypothermia and inflow occlusion. Studies of the hemodynamic, electrocardiographic and electroencephalographic changes during hypothermia are made during the course of these operations.

Extensive experimental use was made of a technique of open intracardiac surgery. This involved perfusion of the carotid and coronary arteries with oxygenated blood during inflow occlusion. In experimental animals 85% survived right ventriculotomy for thirty minutes. In five clinical trials of the method however, there were no survivors. The method has been discontinued and future work will be directed toward an effective biologic or mechanical extracorporeal circulation.

Most of the projects in the Experimental Surgical Laboratory have been concerned with various aspects of valvular heart disease. Last year an excellent method was developed for the graded production

of aortic insufficiency in dogs. The hemodynamic studies in these animals have been carried to patients with aortic valve disease as described above. It was found that the dogs with aortic insufficiency were extremely susceptible to subacute bacterial endocarditis and 100% of the animals would contract the disease with only a small intravenous inoculum of either streptococcus or staphylococcus. This susceptibility is attributed to the increased cardiovascular stress from the valve lesion. It was found, however, that a Hufnagel artificial valve did not protect against endocarditis in these dogs. Present studies are being carried out relative to the role of the adrenocortical hormones in the susceptibility of these animals to endocarditis.

Several patients have recently been observed with combined aortic insufficiency and mitral insufficiency. There was much conjecture as to the advisability of putting an artificial aortic valve in these patients. Accordingly, ten animals were prepared with the combined lesion and the effect of a Hufnagel valve determined. It was found that the valve strikingly lowered the left atrial mean pressure and the left ventricular end diastolic pressure. This would indicate that patients should benefit from the Hufnagel operation even if mitral insufficiency is present.

The present operations performed for aortic stenosis are done blindly, either through a pouch sutured to the aorta or by means of dilators passed through the left ventricle. It is often difficult to open a badly calcified valve effectively by these techniques. Accordingly, using hypothermia, an attempt has been made to expose the aortic valve for direct-vision operation. It was thought that coronary air embolism would be a serious problem but in more than twenty animals operated upon, this has not been the case. The technique has also furnished an opportunity to produce an experimental valvular aortic stenosis by direct suture of the aortic leaflets. Should the technique prove as safe as it now seems, open operation on the aortic valve will be an important clinical contribution.

Hypothermia is in common clinical use as an adjunct to cardiac surgery. Clinical observations have led to the belief that the hypothermic heart may have a much lower tolerance to digitalis than the normothermic one. Experiments to determine this have been carried out. It was found early that the blood pH was an important factor also and it has been necessary to control the respirations carefully as well as the temperature and digitalis dosage. The studies thus far indicate that the blood pH is probably of more importance than temperature in sensitivity to digitalis.

Present methods of treatment of valvular pulmonary stenosis depend to a certain extent upon destruction of the function of the valve. There has been much controversy as to whether a competent pulmonary valve is important. If it is unnecessary, a more adequate operation could safely be performed to relieve pulmonary stenosis. Accordingly, the pulmonary leaflets in dogs have been completely ex-

cised and the animals are being observed for physiologic changes. There have been no deleterious effects noted and right heart pressures have remained normal for a study period of four months. Prolonged observation will be necessary, however, in these animals.

All methods of extracorporeal circulation must provide for perfusion of the coronary arteries during the period of inflow occlusion. There has been much discussion about the optimal amount of blood that should be perfused through the coronary arteries in this situation. Too low a flow results in ventricular fibrillation, while too great a flow causes cardiac dilatation and failure. By determination of coronary sinus flow and oxygen content, a figure of 2 cc./Kg. of body weight/min. has been arrived at as optimal. Further experiments are in progress to determine the validity of this figure and the effects of higher rates of flow.

NATIONAL HEART INSTITUTE

EXTRAMURAL ACTIVITIES

Research Grants

During the fiscal year 1955, research projects supported by grant funds have continued the search for the underlying causes of cardiovascular disease as well as the development of more effective methods for diagnosis and treatment. This is illustrated in the multiphasic attack on arteriosclerosis from which it is becoming increasingly evident that the occurrence and progress of the disease are influenced by internal factors such as the balance or imbalance of hormones from the various endocrine glands and by external factors such as the amount of cholesterol and other fats in the diet. In order to increase the effectiveness of the research and the speed with which results can be obtained, there is arising a form of cooperation between investigators in two or more institutions, whereby a diversity of skills and combination of effort are brought to bear on the solution of a problem. Surgeons concerned with the correction of cardiovascular defects or injuries are continuing their efforts to secure long-lasting grafts in diseased blood vessels, to provide more adequate repair of heart abnormalities and to devise an efficient method for increasing the blood supply to the heart following coronary thrombosis. The part played by other organs in cardiovascular disease is recognized by the increase in research on the inter-relationships of function in the heart, lungs, and kidneys.

The following projects carried on during the past year may be cited among those which show particular promise. Dr. C. J. Wilhelmj and his collaborators at Creighton University in Nebraska have found that high protein or high carbohydrate diets, alternating with periods of fasting, did not permanently affect the blood pressure of normal dogs but that high fat diets under the same conditions appeared to render the animals potentially hypertensive. A study of fourteen angina patients from the University of Pennsylvania Hospital, conducted by Dr. Peter Kuo and Dr. C. R. Joyner (a former Heart Institute trainee) has indicated that typical angina attacks developed following a high fat meal and coincided with the time when fatty materials in the blood were at their peak. The influence of the endocrine glands on the nature of the fats within the blood is substantiated by the research of Drs. Russ, Eder, and Barr at the New York Hospital-Cornell Medical Center. They have found that estrogen, the female sex hormone, tends to restore the normal lipid pattern in human males who have survived a heart attack by reducing the high level of the lipoproteins which are characteristic of this condition. On the other hand, the administration of additional testosterone, the male sex hormone, increases the amount of these lipoproteins in persons whose blood lipids had previously been normal. Evidence that hypertension and coronary artery disease may run in families has been

offered by a study of the families of 266 Johns Hopkins medical students. Statistical data accumulated by Dr. C. B. Thomas and B. H. Cohen indicate that the greatest proportion of affected persons was found among the offspring of two affected parents. The data suggest, also, that the origin of these diseases is complex and may involve a number of genetic factors which can be modified by environmental conditions. A research program which focuses attention on the underlying metabolic derangements of the muscle in heart failure is being carried on by Dr. W. Raab of the University of Vermont. He has found that the efficiency of heart muscle depends on the mutual interplay and balance of nervous and hormonal stimulation and that epinehprine, a hormone which acts as a heart muscle stimulant, actually impairs efficiency by increasing oxygen consumption. Thus, its accumulation within the heart may be a contributing factor in the development of heart failure. Dr. E. S. Benson of the University of Minnesota has found that the contractile substance of the muscle itself is altered in failing hearts. The involvement of the lung in cases of heart disease is illustrated in the research of Drs. R. M. O'Neal and W. A. Thomas at Washington University School of Medicine. They found that individuals with certain cardiac anomalies developed pulmonary arteriosclerosis. A large percentage of the arterial lesions were associated with thrombi which appeared to be incorporated into the walls of the arteries in the development of the sclerotic area. When pulmonary hypertension occurred, the severity of the lesions was increased. One of the problems associated with the repair of blood vessels is related to the interaction between the tissues of host and graft. A team of investigators at Baylor University College of Medicine and the Veterans Administration Hospital in Houston, Texas, produced hypercholesteremia experimentally in dogs which had previously received a graft within the aorta. It was found that atherosclerotic lesions developed in the graft as well as in the rest of the aorta in those animals with serum cholesterol levels in excess of 1,000 milligrams per 100 cubic centimeters of blood; in animals with lower serum cholesterol levels, the graft was unaffected although lesions might be present in other parts of the aorta. From the many drugs which have been tested for the relief of hypertension, Drs. A. Agrest and S. W. Hoobler at the University of Michigan find that pentolinium tartrate (Ansolysen) seems promising for long-term therapy if the dosage is carefully regulated to the individual needs of the patient.

Training Grants

The training grant program provides for the establishment of needed research and health training programs in the cardiovascular and related areas. It assists in the research training of competent young people in the fundamental and applied medical sciences and encourages the training of physicians, nurses and other personnel in the application of the latest available clinical information to the

cardiovascular diseases. These objectives are attained by providing undergraduate and graduate training grants for the establishment and improvement of facilities in carefully selected institutions, including the payment of indirect traineeship and fellowship awards, and by the awarding of direct National Heart Institute traineeships to selected applicants.

During the fiscal year 1955, undergraduate training grants were made to 76 four-year medical schools, 3 two-year medical schools, and 6 schools of osteopathy. Training grants were made, also of each of the 10 schools of public health to assist in the development of their chronic disease training programs. Twelve graduate research training grants and 3 graduate clinical training grants were awarded to aid in the development of graduate cardiovascular research and clinical training programs. Fifteen training grants were made in the area of rehabilitation.

Traineeships designed to permit qualified individuals to receive training in the diagnosis, prevention, and treatment of cardiovascular disease were awarded to 105 physicians in 13 institutions located in 23 states, the District of Columbia, England, Sweden, Puerto Rico, Brazil, and Scotland. Of this number, 13 had requested renewal of their traineeships and were granted another period of training.

Research Fellowships

These grants are awarded to qualified men and women who wish to secure research training in the cardiovascular and related fields. There is an increasing demand for research training by physicians and scientists who wish to learn new techniques and new research concepts in order that they may be applied to areas of cardiovascular concern. During the fiscal year 1955, 84 predoctoral, 136 postdoctoral, 16 special, and 24 medical student part-time fellowships were awarded.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE OR OTHER NIH UNIT

2. Administration
LABORATORY OR BRANCH

3. _____
SECTION

4. _____
LOCATION (IF OTHER THAN BETH.

5. NHI-1
SERIAL NO.

6. Office of the Director, National Heart Institute
PROJECT OR ACTIVITY TITLE

7. Dr. James Watt, Director: Robert H. Grant, Excutive Officer
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. None
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

The Office of the Director is responsible for the planning, development and management of the total Institute program involving $18. million during fiscal year 1956. The activities of the Heart Institute is nationwide in its scope and is concerned with four major areas: (1) extramural programs (Grants and Training), (2) intramural programs (laboratory and clinical research), (3) technical services (Heart Information, Biometrics, and Epidemiology and (4) technical assistance to States (assisting states and other agencies in the use of the most effective methods of prevention and treatment of heart disease).

This Office is responsible for the coordination of the total program involving over 450 positions toward a common goal of improving the health of the people of the United States through these various functions in relation to cardiovascular disease.

R.P.C. - 2 Analysis of NIH Program Activities
December 1955

Budget Data Sheet

10. NIH-1
SERIAL NO.

11. BUDGET DATA:

12. BUDGET ACTIVITY:

Administration

13. IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S).

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

None

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-1
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Technical Services Branch — LABORATORY OR BRANCH
3. Heart Information Center — SECTION
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-2 — SERIAL NO.
6. Heart Disease Information Program — PROJECT TITLE
7. Lealon E. Martin; Carl R. Strom; Evelyn T. Van Steenberg — PRINCIPAL INVESTIGATOR(S)
8. Edward Grover, Louise Young, Arthur Cosing, Robert Palmer, Robert Hinkel, Elizabeth Ingram, — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION

Project:

Carry out a program of professional and public information on heart disease, as specified in the National Heart Act.

Objectives:

To provide information on all aspects of heart disease; to promote increased use of present knowledge and research findings; to assist prevention, early case-finding, diagnosis, and treatment of heart disease through lay education; and to keep individuals and organizations in the heart disease field informed as to current developments and the experience of others.

Methods Employed:

A wide range of media of communication are utilized. Activities include the preparation and distribution of heart disease information-education articles, leaflets, pamphlets, and other items; planning and development of audio-visual materials, exhibits, radio and television broadcasts and transcriptions; preparation of reports and other special materials; issuance of releases and providing assistance to the press, periodicals, and science writers; maintenance of an inquiry and reference service; and cooperation with other agencies and organizations in planning and executing informational and educational projects on diseases of the heart and blood vessels.

Major Findings:

Among the many information-education materials produced and activities conducted during 1955 were:

Exhibits on 1) the Programs of the National Heart Institute--presented at the Americal Medical Association, American Heart Association, and other major medical meetings, and 2) professional consultation and assistance to NHI scientists and others on exhibits and exhibit techniques.

Reports such as Highlights of 1955 Heart Research Progress; Research Progress Reports summarizing advances in understanding drugs, surgery for acquired heart disease, high blood pressure, etc. Items such as administrative reports, budgetary statements, and other special materials were prepared in whole or in part.

Publications. Publications were issued in 1955 in connection with the campaign for prevention of rheumatic fever and rheumatic heart disease. These included a leaflet for the general public, "Stop Rheumatic Fever", a film discussion guide for use at public meetings, and other program materials.

Audio-visuals. A new motion picture, "Stop Rheumatic Fever", was produced and released early in 1955 for nation-wide health education use. The film shows that rheumatic fever can be prevented by treatment or prevention of strep infections. It is part of a health education unit developed by the Heart Institute and the Heart Disease Control Program (DSHS) in cooperation with the Council on Rheumatic Fever and Congenital Heart Disease of the American Heart Association. Initial distribution of the film to health departments was made by the Public Health Service. The Heart Association is cooperating in general distribution; the film was made available for purchase at an unprecedented low cost; and some 800 prints were in use by end of 1955.

Releases and services to writers were important facets of public information activities. In addition to regular releases, such as those reporting grants and awards, lecture announcements, or newsworthy appointments, some 26 special releases were made to professional journals and science writers on specific research accomplishments by Institute scientists. In addition, many services were given newspapers, magazines, television, radio, and other media of mass communication, through working directly with writers, reporters, and others. As a result, articles on heart subjects citing NHI work appeared in a number of large-circulation outlets.

Informational services were also furnished in connection with various meetings.

Heart Research News, for exhibit and administrative use, was begun in 1955 and will be continued in 1956. Consisting of brief items of interest on published heart research work, it was exceptionally well received.

Inquiry and reference service was provided as a continuing and increasing function in meeting mail, telephone, and in-person requests for information on heart disease subjects from the general public, individuals in medical and health professions, and organizations. Inquiries not answerable by forwarding of suitable publications or other already available material required special compilation of references or information.

Special Programs:

Rheumatic Fever:

Development of a nationwide campaign for rheumatic fever prevention with initiation of an intensified professional and public educational program beginning in January and continuing as a major activity of the year; establishment of this campaign as a joint program with American Heart Association and Heart Disease Control Program (DSHS); implementation of the campaign through NIH, PHS, and DHEW support and endorsement; assistance in implementing other aspects of this program including the first distribution of educational material to all physicians and medical students ever participated in by PHS.

Cerebral Vascular Diseases:

Planning and development of ideas and materials for programs in this field which, continuing next year, should lead to production and use of much-needed educational materials as well as stimulation of other activities. A new booklet for the public was developed during 1955, for issuance in 1956. It will be the first such publication in this field and wide distribution and use is anticipated.

Heart Disease:

Development of a new booklet on normal heart and circulation and their diseases. Containing functional illustrations, it will meet need for such a publication, will be first of its kind issued, and should be extensively disseminated.

Significance to HEART Research: (Name of Institute)	Significance to HEART Research:
(Note: The significance of H.I.C. activities to public health heart programs, which is also of major importance, is not included per se in the report form, but some indication of this aspect of H.I.C. responsibilities can be seen in various parts of this report.)	Provides information concerning research development and advances; assists the dissemination of new knowledge; furthers the use of research findings; furnishes consultation and services as needed in preparation of exhibits and other graphies, in clearance and printing procedures, and other matters involving media of communication.

Proposed course of project:

This is a continuing operation with its general function specified by law. Informational activities will continue to be conducted in response to needs, with variance in emphasis, content, or media as indicated by program needs. In the coming year, the rheumatic fever program, will receive major emphasis. Also, cerebral vascular diseases, hardening of the arteries, and normal physiology will be stressed. Improvement and expansion of public reporting of research is also planned for the year.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-2
SERIAL NO.

11. BUDGET DATA:

12. BUDGET ACTIVITY: Research

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH)

None

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-2
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

See under 9.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Lealon E. Martin -- NIH Nominee, Rockefeller Public Service Awards; Member, Board of Directors; Montgomery County TB and Heart Association; Chairman, Committee in Humanities, NIH Graduate School.

Mrs. E. T. Van Steenberg -- Winner NIH Cash Award for Superior Performance.

R.P.C. -1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Technical Services Branch — LABORATORY OR BRANCH
3. Biometrics — SECTION
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-3 — SERIAL NO.

6. Statistical Consultation and Research Services — PROJECT TITLE

7. Dr. Max Halperin, Felix E. Moore — PRINCIPAL INVESTIGATOR (S)

8. _____ — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION: Provision of consultative services in statistical design and analysis of experiments, with associated research in statistical methodology.

(A) Objectives: The primary objective of this service is to provide assistance in the statistical design, interpretation and evaluation of experimental studies conducted by the scientific staff of the National Heart Institute, and by grantees of NHI.

(B) Methods employed: The assistance described above is accomplished using the standard methods of biometry, statistics and probability, with necessary modifications as required by the problem at hand. Not infrequently standard statistical methods are not available to handle research problems, and it is necessary for personnel of the section to develop new methods.

(C) Major findings: As a service organization, this section primarily assists other investigators in making important biological and medical findings, rather than in making such findings directly. Such assistance is frequently acknowledged in the publication of findings of the principal investigators. Occasionally, the nature of the assistance is such as to warrant co-authorship rather than simple acknowledgement.

(D) Significance for Cardiovascular Research: This is a specialized service which is provided as requested by investigators. As such it makes a general contribution to the increase of the research potential.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10 NHI-3
SERIAL NO.

11
BUDGET DATA:

12
BUDGET ACTIVITY:

RESEARCH (SERVICE) /X/ ADMINISTRATION / /

REVIEW & APPROVAL / / TECHNICAL ASSISTANCE / /

13
IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S)

None

14.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSE WHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIH-3
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Halperin, M., Greenhouse, S.W., Cornfield, J., and Zalokar, J : "Tables of Percentage Points for the Studentized Maximum Absolute Deviate in Normal Samples". Journal of the American Statistical Association, (March, 1955)

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R.P.C. -1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Technical Services Branch
LABORATORY OR BRANCH

3. Biometrics
SECTION

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. NHI-4
SERIAL NO.

6. Statistical services for Cooperative Study of Lipoproteins and Atherosclerosis
PROJECT TITLE

7. Felix E. Moore, Chief, Tavia Gordon
PRINCIPAL INVESTIGATOR(S)

8. _____
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

(A) Objectives: To assess the value of various measures of lipoproteins and cholesterol in predicting development of atherosclerotic disease; to measure the association of elevated lipoprotein with various diseases; to provide standard lipoprotein measurements values for normal populations.

(B) Methods employed: Four laboratories (at the University of California, Cleveland Clinic, University of Pittsburgh, and Harvard University) have cooperated to secure blood samples from large, representative population groups. Lipoprotein measurements (by the ultracentrifuge) and cholesterol measurements (by the Abell-Kendall method) have been done on approximately 10,000 cases. At the end of two years follow-up status is secured for each case. Results are reported to the Biometrics Research Section which acts as statistical coordinator for the group.

(C) Major findings: Final reports will probably be available in the first half of calender year 1956.

(D) Significance to HEART research: Discovery of a test predictive of cardiovascular disease, and one which would permit assessment of measures for the prevention of atherosclerosis would be of great practical value. This study is teating a procedure proposed for that purpose.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-4
SERIAL NO.

11. _____
BUDGET DATA:

12. _____
BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. _____
IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S)

Donner Laboratory, University of California; Cleveland Clinic, Cleveland, Ohio; Department of Biophysics, University of Pittsburgh; Department of Nutrition, School of Public Health, Harvard University. The study in these institutions is financed from research grant funds administered by the National Heart Institute.

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-4
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

R.P.C. -1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Technical Services Branch (LABORATORY OR BRANCH)

3. Biometrics (SECTION) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NHI-5 (SERIAL NO.)

6. Development and Application of Statistical Methods to the Study of Cardiovascular Disease in Human Populations (PROJECT TITLE)

7. Felix E. Moore, Tavia Gordon (PRINCIPAL INVESTIGATOR(S))

8. All professional personnel of Section (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

(A) Objectives: To seek out and create sources of data on the morbidity and mortality from cardiovascular diseases; to analyse these for the information they provide on the extent of the problem, on the trends in time, and differentials among the age, sex, geographic groups; to explore epidemiological leads provided by these analyses; to prepare materials for dissemination to professional and lay groups in cooperation with associated specialists.

(B) Methods employed: Available materials are collected from such sources as the national offices of vital statistics (U.S. and foreign), the various collectors of routine morbidity statistics (eg. Armed Forces), and the various groups carrying on ad hoc morbidity surveys (California, Baltimore, Hunterdon County, etc.). In addition, the Section cooperates with groups engaged in special field surveys, assisting them in their planning to the end that needed data can be secured.

(C) Significance to HEART research: This activity provides investigators with information required as a background for their work, and may provide leads for further laboratory, clinical or field investigation.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-5
SERIAL NO.

11. _____
BUDGET DATA:

12. _____
BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. _____
IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S)

14. _____
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-5
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE OR OTHER NIH UNIT
2. Technical Services Branch — LABORATORY OR BRANCH

3. Biometrics — SECTION
4. — LOCATION(IF OTHER THAN BETH.)
5. NHI-6 — SERIAL NO.

6. Study of mortality and disability retirement among railroad workers
PROJECT OR ACTIVITY TITLE

7. Felix E. Moore
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. Dr. William Baum and Mr. William Haenszel, National Cancer Institute
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION: Primary responsibility for this study lies in the National Cancer Institute. NHI is providing some funds and will share in analytical responsibility.

Objectives:

To investigate differences in cardiovascular disease rates which may be associated with differences in occupational groups among railroad workers in the U.S.

Methods Employed:

A cohort of all working and retired railroad employees with at least ten years' exposure in the industry as of January 1, 1953 will be set up (approximately 1,000,000 persons). From its records the Railroad Retirement Board can assemble the population base data and information on mortality and disability retirement for this cohort. A procedure has been worked out to assemble the information from the various sources and reproduce it on a special set of study punch cards, which can be placed at the disposal of the investigators. Sufficient identifying detail has been provided to make possible further studies of groups exhibiting unusually high rates. The Railroad Retirement Board under contract has supplied punch cards for the cohort understudy and is now searching their records and preparing reports on mortality and disability retirement for the second study year.

Significance to Heart Research:

Railroad employees are an occupationally stable group, with their own retirement system. This is an ideal setting in which to attempt investigation of occupational factors and will provide good experience for later handling occupational data collected in Social Security Board operations. Differences in occupational groups are large enough so that the effort hypotheses can be tested.

Proposed Activities During the Next Calendar Year:

At least two years mortality experience need to be collected before findings on occupational differentials and mortality can be reported. It is anticipated that mortality and disability retirement experience may be collected over a period of years and that details on specific occupations and exposures to forces of suspecting of influencing cardiovascular diseases.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-6
SERIAL NO.

11. BUDGET DATA:

12. BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S)

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIH-6
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE OR OTHER NIH UNIT

2. Technical Services Branch
LABORATORY, BRANCH OR DEPARTMENT

3. Biometrics
SECTION

4.
LOCATION (IF OTHER THAN BETH)

5. NHI-7
SERIAL NO.

6. Statistical studies related to Framingham Heart Disease Epidemiology Study
PROJECT OR ACTIVITY TITLE

7. Felix E. Moore, Chief
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. Dr. T.R. Dawber, Director, Framingham Heart Disease Epidemiology Study
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

This project is described in detail in the Project Description sheet submitted for the Epidemiology Section, Technical Services Branch, NHI.

The Biometric Research Section takes responsibility for (a) general statistical planning for the study, (b) final preparation of forms, (c) development of coding procedures, (d) planning of punch card methods and liaison with NIH Statistical Processing Section, (e) preparation of statistical analyses for reports in collaboration with staff of the Epidemiology Section.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-7
SERIAL NO.

11. BUDGET DATA

12. BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S)

None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH

None

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIH - 7
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955

None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Technical Services Branch — LABORATORY OR BRANCH
3. EPIDEMIOLOGY — SECTION
4. Framingham, Mass. — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-8 — SERIAL NO.
6. Heart Disease Epidemiology Study — PROJECT TITLE
7. Thomas R. Dawber, Medical Director — PRINCIPAL INVESTIGATOR
8. George V. Mann. Surgeon (R); Abraham Kagan, M. D. (Civil Service); George Cytroen, Sr. Asst. Surgeon (R) Cardiologist — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Heart Disease Epidemiology Study, Framingham, Massachusetts

Objectives: The objective of this project is 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.

Methods Employed: In 1950, by random sampling method, 6,353 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. Those were all given initial examinations with the view toward doing re-examinations at two-year intervals over a long period, during which all the data of pertinent constitutional or conditioning factors will be recorded and analyzed.

Major Findings: In 1954, considerable progress was made in the second, third and fourth examination of the population group under study. The cumulative number of persons examined as of December 31, 1955 is as follows:

EXAM	TOTAL	SAMPLE	SX
I	5234	4494	740
II	4822	4082	740
III	3886	3405	481
IV	1465	945	520

9. Major Findings; (Continued): The project has been set up as a continuing one since it will take a prolonged period of time before major epidemiological findings become available. At present, data on prevalence and incidence of cardiovascular disease are being analyzed.

Significance to Heart Research: In order to develop an understanding of the way in which arteriosclerotic and hypertensive heart disease develop it is necessary to obtain vital information regarding the life history of these diseases by observing how and where and in what way they develop. The type of epidemiologic research done by this project will provide this type of information.

Proposed Course of Project: It has always been recognized that the continued cooperation of the population was the first essential if this project were to be successful. All attempts have been continually made to keep the enthusiasm of the participants at a high level. This involves a number of factors primarily associated with the conduct of the interview and examinations and with personal reassuring of the participants as to the part which they are playing in this type of research. It also involves keeping in frequent contact with those who have moved away.

A measure of the success of these efforts is the percentage of successful follow-up which, at the close of 1955, was 95.6% for both the third and fourth re-examinations.

Another measurement which may be applied is that of success in obtaining later cooperation of individuals who have declined a previous examination. Of the 423 subjects who for various reasons did not appear for Exam II, to date 112 have returned for either Exam III or Exam IV. It is believed that continued effort in this direction will eventually bring back a high percentage of this group.

Considerable progress has been made in supplementing our records with additional information about illnesses and hospitalizations of the subjects under study. A high degree of cooperation of outside institutions has been encountered in this work.

Plans are under way to do an intensive study of the dietary habits of the population, in the fifth phase of the study which begins in October, 1956. Planning is also under way for the possibility of making an evaluation of emotional status and of hormonal factors in subsequent examinations. In this way it is hoped to obtain a measurement of as many factors as possible which may have some bearing on the development of cardiovascular disease.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-8
SERIAL NO.

11. _____
BUDGET DATA:

12. _____
BUDGET ACTIVITY:

RESEARCH (SERVICE) [X] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [ ]

13. _____
IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S)

14. _____
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-8
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Superior Performance and Adopted Suggestions:
John W. Claffey, X-Ray Technician, NHI

R.P.C. -1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Grants and Training Branch — LABORATORY OR BRANCH
3. _____ — SECTION
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-9 — SERIAL NO.

6. Review and Approval of Research and Training Grants — PROJECT TITLE

7. Dr. J. Franklin Yeager — PRINCIPAL INVESTIGATOR(S)

8. Dr. Murray Goldstein, Dr. Helen Kaan, Kathleen Harlow, Jeanne Walton, Elva Hershey — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION (SEE INSTRUCTIONS AND SAMPLES)

Research Grants

An appropriation of $8,550,000 was available for research project grants in the fiscal year 1956. As of January 1, 1956, 733 research grant applications were recommended favorably by the National Advisory Heart Council in the amount of $9,425,363 and 637 were awarded in the amount of $8,420,936. It is expected that there will be 182 new applications presented for consideration at the February meeting of the Council. The funds available at that time exclusive of possible savings, $129,064, will provide for the payment of approximately 12 additional research grants.

Training Grants

$3,142,000 was appropriated in the fiscal year 1956 for research and clinical training grants at the undergraduate and graduate level and for traineeship awards. As of January 1, 1956, training grants were made to 98 institutions in the amount of $2,510,000; commitments have been made in the amount of $93,814 for the payment of training grants to 4 additional institutions, leaving a balance of $71,573 for new grants. It is expected that there will be 9 new training grant applications presented for consideration by the National Advisory Heart Council at its February 1956 meeting. If these are recommended for approval, the funds currently available can provide for the payment of 3 grants. Traineeships in the amount of $378,993 have been awarded to 95 individuals during the fiscal year. There are presently available $87,522, which will permit the payment of approximately 15 additional traineeships following their favorable recommendation by the National Heart Institute Traineeship Board.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10 NHI-9
SERIAL NO.

11.
BUDGET DATA:

12.
BUDGET ACTIVITY:

RESEARCH (SERVICE) [ ] ADMINISTRATION [ ]

REVIEW & APPROVAL [X] TECHNICAL ASSISTANCE [ ]

13.
IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S)

None

14.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

None

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Pbulications Sheet

15. NHI-9
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Research
LABORATORY OR BRANCH

5. NHI-10
SERIAL NO.

6. Office of the Associate Director(In Charge of Research), National Heart Institute
PROJECT TITLE

7. Dr. Robert W. Berliner
PRINCIPAL INVESTIGATOR(S)

8. J. F. Monahan
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

The Office of the Associate Director (In Charge of Research) is immediately responsible for the overall planning and direction of the direct research program (laboratory and clinical investigations) of the National Heart Institute, involving over $4 million and a budgeted staff of 328 for Fiscal Year 1956. This entails determination of the scope of the research program; selection, organization, and provision of leadership to the professional staff; and review and evaluation of the results of research activities. Continuous study of national and inter-national progress in the field of cardiovascular research is carried out, in order to make certain that the research program of the Institute is of maximum effectiveness in developing knowledge of the causes and methods for prevention and treatment of the cardiovascular diseases.

10. NHI-10
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. None

14. None

Honors, Awards, and Publications

15. NHI-10
SERIAL NO.

16. None

17. None

ANALYSIS OF NIH PROGRAM ACTIVITIES

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Kidney & Electrolyte Metabolism — LABORATORY OR BRANCH
3. None.
4. None.
5. NHI-11 — SERIAL NO.
6. Mechanisms of Fluid and Electrolyte Retention in Experimental Cardiac Failure. — PROJECT TITLE
7. James O. Davis — PRINCIPAL INVESTIGATOR (S)
8. Wilmot C. Ball, Jr., M. Jay Goodkind and Lala Mathers Dunbar. Also Maurice M. Pechet for Projects I and II. — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION:

PROJECT I

Project: Increased aldosterone excretion in urine from dogs with cardiac failure and from dogs with ascites secondary to thoracic vena cava constriction.

Objective: To examine urine from experimental animal preparations showing chronic Na retention for the presence of a Na retaining substance and to identify the chemical nature of the material.

Methods Employed: Urine is extracted with an organic solvent, methylene chloride. The solvent is evaporated and the residue dissolved in alcohol. The extract is injected into adrenalectomized assay dogs to characterize the effect on Na and K excretion. Potency is determined from a dose response curve in the same adrenalectomized assay dogs. The source of the active agent is determined by removal of the adrenal glands. Chromatographic fractionation and assay is used to identify the Na-retaining substance.

Major Findings: The Na retaining substance in urine is aldosterone, the naturally occurring Na retaining hormone of the adrenal cortex. The hormone is present in large amounts in urine from dogs with cardiac failure and from dogs with caval constriction whereas no hormone is detectable in the same quantity of normal dog urine.

Significance to Heart Research: To treat congestive heart failure in the most effective manner it is necessary to understand the physiology of the syndrome. The recognition that Na retention in heart failure is due to increased circulating aldosterone points to the need for a potent antihormone to counteract aldosterone. The finding of increased

amounts of aldosterone in urine of the dog with experimental cardiac failure provides an excellent experimental animal for further studies to determine the source of the high blood level.

Proposed Course of Project: To determine whether increased circulating aldosterone results from (1) increased secretion by the adrenal cortex, (2) decreased degradation by the liver or (3) a combination of both.

PROJECT II

Project: Increased secretion of aldosterone by the adrenal cortex in dogs with cardiac failure and in dogs with thoracic inferior vena cava constriction and ascites.

Objectives: To determine whether aldosterone is secreted at an increased rate in dogs showing chronic Na retention.

Methods Employed: The concentration of aldosterone is determined in adrenal vein blood from (1) normal dogs, (2) dogs with heart failure and (3) dogs with thoracic inferior vena cava constriction and ascites. Also, the rate of flow of blood from the adrenal gland is determined so that the minute output of aldosterone can be calculated. To determine the concentration of aldosterone in adrenal vein blood, the blood is extracted and chromatographed; all chromatographic fractions are assayed for aldosterone-like activity.

Major Findings: Preliminary observations indicate increased secretion of aldosterone in both dogs with heart failure and dogs with thoracic caval constriction and ascites.

Significance to Heart Research: This finding provides a very important link in the chain of events from the heart to the kidney in elucidation of the mechanisms of Na retention in congestive heart failure.

Proposed Course of Project: The possibility of a decreased rate of degradation of aldosterone by the liver as a secondary mechanism for increased circulating aldosterone must be investigated. The role of the anterior pituitary gland and of the hypothalamus in activation of the adrenal cortex to secrete aldosterone will be studied.

PROJECT III

Project: Effect of hypophysectomy on aldosterone excretion in urine from dogs with thoracic inferior vena cava constriction and ascites.

Objectives: To determine the role of the anterior pituitary gland in the control of aldosterone production.

Methods Employed: The urinary excretion of aldosterone was studied by the methods in Project I. Dogs with thoracic vena cava constriction and ascites were subjected to total hypophysectomy and the urinary output of aldosterone and sodium was followed for several weeks.

Major Findings: A marked drop in the urinary excretion of aldosterone occurred. Concurrently, an increase in sodium excretion resulted.

Significance to Heart Research: The present data provide information on the relation of the anterior pituitary gland to aldosterone excretion in urine from dogs with experimental ascites. The findings also furnish valuable information on the fundamental relationship of the pituitary to the adrenal cortex.

Proposed Course of Project: During the next few months it is planned to administer ACTH to hypophysectomized dogs with caval constriction in an attempt to reverse the changes in sodium exoretion and aldosterone output. Also, it is planned to reconstrict the thoracic inferior vena cava in the hypophysectomized dog with caval constriction in order to increase venous pressure to the pre-hypophysectomy level.

PROJECT IV

Project: Effect of hemorrhage on the urinary excretion of aldosterone in dogs.

Objectives: To investigate the role of aldosterone in the retention of sodium following hemorrhage.

Methods Employed: Same as for Project I.

Major Findings: An increase in aldosterone-like activity in urine was associated with sodium retention following hemorrhage.

Significance to Heart Research: The data show the important role of aldosterone in homeostasis.

Proposed Course of Project: It is planned to determine the various factors which influence the adrenal cortex to secrete increased amounts of aldosterone following hemorrhage. The importance of decreased sodium intake and of decreased plasma protein will be evaluated.

PROJECT V

Project: Ascites formation without sodium retention in dogs with thoracic inferior vena cava constriction and in dogs with pulmonary artery constriction.

Objectives: To determine if ascites will form in the absence of sodium retention by the kidney.

Methods Employed:

1. Measurement of ascitic fluid volume by T-1824 dye dilution.
2. Metabolic balance measurements for sodium and potassium.
3. Plasma electrolyte determinations by flame photometry.
4. Urinary excretion of aldosterone by methods described in Project I.

Major Findings: The major finding of this project was that ascites formed in the absence of sodium retention by the kidney.

Significance to Heart Research: This study provides data which are consistent with the hypothesis that sodium retention by the kidney is initiated by a backward failure mechanism.

Proposed Course of Project: This project is definitive.

ANALYSIS OF NIH PROGRAM ACTIVITIES

Budget Data Sheet

10. NHI-11
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S).

Project II. Increased secretion of aldosterone by the adrenal cortex in dogs with cardiac failure and in dogs with thoracic inferior vena cava constriction and ascites.

This project is being done in collaboration with Dr. Maurice Pechet of Harvard Medical School.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH).

This research parallels research conducted in patients on the physiology of aldosterone in relation to congestive heart failure and cirrhosis of the liver by Doctors Grant W. Liddle, Leroy Duncan and Fred Bartter.

ANALYSIS OF NIH PROGRAM ACTIVITIES

Honors, Awards and Publications Sheet

15. NHI-11
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FOR THIS PROJECT DURING CALENDAR YEAR 1955:

1. Davis, James O., Hyatt, Robert E. and Howell, David S.: Right-sided congestive heart failure in dogs produced by controlled progressive constriction of the pulmonary artery. Circulation Research 3:252, 1955.

2. Davis, James O., Howell, David S. and Hyatt, Robert E.: Effects of ascites and chronic digoxin administration in dogs with right-sided congestive heart failure produced by pulmonary artery constriction. Circulation Research 3:259, 1955.

3. Howell, David S., Davis, James O. and Laqueur, G. L.: Effect of hypophysectomy on electrolyte excretion in dogs with ascites produced by thoracic inferior vena cava constriction. Circulation Research 1:264, 1955.

4. Davis, James O., Howell, David S. and Hyatt, Robert E.: Sodium excretion in adrenalectomized dogs with chronic cardiac failure produced by pulmonary artery constriction. Am. J. Physiol. 183:263, 1955.

5. Davis, James O., Howell, David S., Goodkind, M. Jay and Hyatt, Robert E.: Accumulation of ascites during maintenance of adrenalectomized dogs with thoracic inferior vena cava constriction on a high Na diet without hormone therapy. Am. J. Physiol. (Accepted for publication).

17. None.

ANALYSIS OF NIH PROGRAM ACTIVITIES

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Kidney & Electrolyte Metabolism
LABORATORY OR BRANCH

3. None

4. None

5. NHI-12
SERIAL NO.

6. Investigation of kinetic processes across ion selective membranes.
PROJECT TITLE

7. Dr. Melvin Gottlieb
PRINCIPAL INVESTIGATOR (S)

8. None.

9. PROJECT DESCRIPTION:

Objectives: The investigation of the kinetics of ionic movement across artificial membranes bearing fixed charges, and the correlation of the ionic fluxes with independently measurable quantities such as the mobilities of ions within the membranes, the ionic selectivity and the electrical resistance of the membranes and the electrical potential arising across the membrane.

Methods Employed: The membranes used are either almost completely anion permeable or almost completely cation permeable. The mobilities of ions within the membrane are calculated from the rates of self-exchange of ions for their isotopes using radiotracers, the selectivity of the membranes among the various permeating ions from the electrical potential arising across a membrane separating solutions of these ions at the same concentration, and the rate of movement of ions across membranes followed by chemical analysis of the solutions separated by the membrane. Electrical potentials and alternating current resistances are measured by standard techniques.

Major Findings: It has been found that the rate of self-exchange of an ion for its isotope across the membrane can be semi-quantitatively predicted from the electrical resistance of the membrane, using the Nernst-Einstein equation relating diffusion coefficients to ionic equivalent conductances, for the several types of cation permeable membranes studied and for anion permeable membranes prepared by incorporating the basic protein, protamine, in collodion. Anion permeable membranes prepared with strong base polyelectrolytes, e.g. polyvinyl-N-methyl pyridinium bromide, had electrical resistances up to ten times higher than predicted from the rates of self-exchange of ions across them. The discrepancy between theoretical and calcu-

lated resistances is independent of the permeating and non-permeating ions present, but increases sharply as the solution bathing the membrane is diluted. The rates of exchange of one species of permeating ion for another across ion selective membranes agrees with the predictions of a treatment of the subject which implicitly ignores the relative chemical specificity of the membrane for the various ions. The rate of exchange of one ion for another across an ion selective membrane may then be calculated from the individual ionic self-exchange rates.

Significance to Heart Research: The understanding of ionic movement across inert, well characterized, charged membranes in relatively simple situations is a necessary preliminary to an understanding and interpretation of movement across the much more complicated biological membranes.

Proposed Course of Project: It is planned to extend the studies on the exchange of one ion for another to include a large number of pairs of ions in order to fully explore the role of membrane selectivity among ions in the kinetics of ion movement. The exchange studies will be expanded to include mixtures of ions and exchange in a concentration gradient. It is hoped to eventually treat the kinetics of the establishment of Donnan Equilibria in terms of independently measurable properties of the membranes.

The study of the anomalous lack of correlation between electrical resistances and rate of isotopic self-exchange across the strong base type anion permeable membrane is being continued with an investigation of the transition at the membrane-solution interface.

ANALYSIS OF NIH PROGRAM ACTIVITIES

Budget Data Sheet

10. NHI-12
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.

Dr. J. Wagner, Visiting Scientist in the Laboratory of Dr. K. Sollner, NIAMD, is collaborating on the aspect of this problem dealing with the anomalous behavious of the strong base polyelectrolyte type membranes.

14. None.

ANALYSIS OF NIH PROGRAM ACTIVITIES

Honors, Awards, and Publications Sheet

15. NHI-12
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

M. Gottlieb, R. Neihof and K. Sollner. Preparation and Properties of Strong Base Type Collodion Matrix Membranes. J. Phys. Chem. In Press.

17. None.

ANALYSIS OF NIH PROGRAM ACTIVITIES

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Kidney & Electrolyte Metabolism
LABORATORY OF BRANCH

3. None

4. None

5. NHI-13
SERIAL NO.

6. Isolation of a Cardiotonic Substance From Mammalian Tissues
PROJECT TITLE

7. Dr. Stephen Hajdu
PRINCIPAL INVESTIGATOR (S)

8. None

9. PROJECT DESCRIPTION:

Objectives: Previous studies on the frog heart suggested the presence of a digitalis-like substance in blood serum. The purpose of this research is to isolate this substance, to characterize it chemically and to determine its role in the control of the contraction of the heart.

Methods Employed: An assay for digitalis-like activity has been developed using the staircase phenomenon of the frog heart. Methods of isolation have involved extraction with organic solvents, chromatography and counter current distribution.

Major Findings: Examination of variety of tissues revealed the following approximate concentrations of the active principle (measured as micrograms equivalents of strophanthidin).

Tissues	Micrograms per Kilo
Adrenal medulla	1000
Adrenal cortex	0
Heart muscle	300
Liver	100
Blood serum	180
Red cells	0
Body muscle	12

Since the active fractions obtained from these sources were chromatographically and biologically indistinguishable, frozen adrenals have been used as the source of material.

The biological importance of the active substance has been further indicated by two observations: (1) It exerts its characteristic cardiotonic effect on the heart of the tropical toad, an organ which is completely insensitive to all known cardiac glycosides. (2) This substance exhibits the characteristic properties of a cardiac glycoside when tested on isolated carotid strips.

The active substance has been obtained in crude form as a fraction occurring in close association with unidentified choline-containing lipids.

Significance to Heart Research: The identification and study of a substance which may play a fundamental role in regulating heart muscle contraction is obviously of the greatest importance to our understanding of the function of the heart.

Proposed Course of Project: Efforts for chemical identification of the active principle are being continued. Further studies of the possible physiological role of the material in the regulation of the heart under normal and abnormal conditions will be undertaken.

3 c

ANALYSIS OF NIH PROGRAM ACTIVITIES

Budget Data Sheet

10. NHI-13
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.

Laboratory of Chemical Pharmacology.

14. None.

ANALYSIS OF NIH PROGRAM ACTIVITIES

Honors, Awards, and Publications Sheet

15. NHI-13
SERIAL NO.

16. None

17. None

ANALYSIS OF NIH PROGRAM ACTIVITIES

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Kidney & Electrolyte Metabolism
LABORATORY OR BRANCH

3. None.

4. None

5. NHI-14
SERIAL NO.

6. Function of Single Nephrons in Necturus
PROJECT TITLE

7. Dr. Thomas J. Kennedy, Jr.
PRINCIPAL INVESTIGATOR (S)

8. Dr. Robert P. Akers
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Project: The study of the operation performed by the nephron in the formation of urine.

Objectives: 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.

Methods Employed: The general technique used is micromanipulative. The nephrons of the kidney of necturus can be visualized with proper lighting and microscopy. Micromanipulators have been built to which micropipettes may be attached for sampling of tubular urine at various levels of the nephron. Segments of the nephron can be isolated between blockades of oil or mercury and the intervening nephron can be perfused. Microelectrodes may be introduced into the tubular lumen for measurement of potential differences across membranes or for the measurement of pH. Ultramicrochemical analytical methods are used to determine the chemical composition of the urine collected from the puncture sites.

Major Findings: To date, most of the effort has been devoted to the fabrication of equipment and to the acquisition of facility in using it. In addition, much time and effort have been devoted to develop-

ment of chemical methodology. Methods for the determination of inulin and chloride on an ultramicro scale have been developed, and in collaboration with Dr. Robert L. Bowman (Laboratory of Technical Development, NHI) instruments for the ultramicro measurement of sodium, potassium and the freezing point of solutions are under development. With the advice and guidance of Dr. Karl Frank (NINDB), apparatus for the measurement of potential differences has been constructed and a few experiments have been performed to document the level of D.C.P.D. across the nephron. Results are at present of a preliminary nature.

Significance to Heart Research: The transport of electrolytes and water across the renal tubule is altered in heart failure. Recognition of the nature and cause of this alteration requires understanding of normal mechanisms of electrolyte transport. Once the normal mechanisms are understood, the possibility of characterization of the abnormalities seen in heart failure becomes more likely. Rational therapy based on sound physiological premises should follow.

Proposed Course of Project: Over the next year the plan is to carry out a number of exploratory experiments and select lines of study that appear, on the basis of preliminary work, most promising. A systematic study of electrical potential gradients along the nephron will be undertaken and the effort thereon of various compounds known to modify electrolyte transport will be studied. In addition, it will be of interest to study simultaneously the relationship between changes in electrolyte transport and D.C. potential changes. If technically feasible, acute changes in the composition of tubular urine will be induced by perfusion of isolated segments of nephron with solutions of composition different from that of glomerular filtrate. A study of the feasibility of insertion of a microglass electrode for pH determination will also be made.

ANALYSIS OF NIH PROGRAM ACTIVITIES

Budget Data Sheet

10. NHI-14
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None.

14. None.

ANALYSIS OF PROGRAM ACTIVITIES

Honors, Awards, and Publications Sheet

15. NHI-14
SERIAL NO.

16. None.

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Kidney and Electrolyte Metabolism
LABORATORY OR BRANCH

5. NHI-15
SERIAL NO.

6. Method for the Evaluation of Diuretics
PROJECT TITLE

7. Renato Kovach (Guest Worker) and Robert W. Berliner
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: This project is concerned with the study of the usefulness of the dog with ascites due to inferior vena cava constriction for the assay and evaluation of diuretic agents.

Objectives: To provide a test object suitable for the assay of diuretics and the evaluation of their usefulness in states associated with edema.

Methods Employed: Dogs are prepared by constriction of the inferior vena cava just above the diaphragm. They are maintained on fixed dietary intake in metabolic cages and all urine is collected periodically. Diuretics are administered and the excretion of fluid and electrolyte measured.

Major Findings: The animals excrete no significant amounts of sodium or chloride spontaneously. When diuretics are administered there is excretion of large amounts of electrolyte and the effect of standard doses of mercurials is adequately reproducible. Initial attempts to do studies involving extended periods of observation have been unsuccessful because of intercurrent illnesses among the animals. Emphasis has been shifted to more short-range experiments which can be completed in a reasonable period.

Significance to HEART Research: The development of new diuretic agents has been severely handicapped by lack of suitable animal preparation for screening and preliminary evaluation, and the usefulness of diuretics in patients with edema has required long periods of experimental trial before even partial determination of their usefulness can be accomplished. The preparation under study in this project promises to serve a very useful purpose in the improvement of therapy in heart disease.

Proposed Course of Project: See above.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-15
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications

15. NHI-15
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Kidney and Electrolyte Metabolism
LABORATORY OR BRANCH

5. NHI-16
SERIAL NO.

6. Effect of Reducing Glomerular Filtration Rate on Renal Function
PROJECT TITLE

7. Douglas G. Davidson (Guest Worker) and Robert W. Berliner
PRINCIPAL INVESTIGATOR(S)

8. Agnes Preston, Doris Woods and Raymond Cherwinski (Technicians)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Effect of reducing glomerular filtration rate on the capacity to produce a dilute urine.

Objectives: To determine the effect of reducing glomerular filtration on the concentration of the urine produced during water diuresis. The information obtained will provide evidence concerning the mechanism by which the urine is diluted and concerning the mechanism of action of antidiuretic hormone. In addition it should provide a background for interpretation of clinical abnormalities of water excretion such as the hyponatremia and impaired water diuresis of cardiac failure and other states associated with edema formation.

Methods Employed: A technique has been developed for acute constriction of the right renal artery in the trained unanesthetized dog undergoing a water diuresis so that the behavior of the normal left kidney serves as a control. The bladder is divided in half so that each half contains one ureteral orifice and the two reformed bladders are attached to the abdominal wall and to the outside through

plastic funnel-clamps. A small inflatable cuff of silicone rubber lining a vitallium clip (devised by Dr. Bowman, Laboratory of Technical Development) is placed on the right renal artery and communicates to the outside through a small polyethylene tube. The cuff is inflated by connecting the tube to a mercury manometer and increasing the pressure.

Major Findings: The results of a large number of experiments in at least 12 different dogs indicate that every measurable reduction of glomerular filtration rate is accompanied by an increase in the concentration of the urine (from the low level characteristic of water diuresis). When filtration rate is sufficiently reduced, the urine becomes hypertonic to blood. Most of the experiments have been done with combined mannitol and water diuresis and under these conditions the reduction of filtration rate required to produce hypertonic urine has been of the order of 70%. Theoretical considerations lead to the inference that the marked degree of constriction necessary to produce hypertonicity is in part attributable to the mannitol included in the infusions. On the other hand omission of non-reabsorbable solute makes the technical problems much greater because of the very low urine flows associated with hypertonic urine and low solute excretion. However, preliminary experiments with low to absent mannitol indicate that 1) much greater degrees of hypertonicity are probably achieved (up to 475 milliosM) and 2) much smaller degrees of constriction suffice for the achievement of hypertonic urines (only 30% reduction of GFR). Anoxia may be excluded as a significant factor in the results of the experiments since the reduction in RPF is relatively small in all experiments and negligible in many.

The results are interpreted to indicate 1) that the urine is diluted by the reabsorption of sodium "salts," 2) that the mechanism which yields a hypertonic urine is not controlled by antidiuretic hormone, 3) that the effect of ADH is on the permeability of the distal tubule to the diffusion of water and that ADH assures delivery to the concentrating mechanism of a fluid essentially isotonic with blood.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-16
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications

15. NHI-16
SERIAL NO.

16. None

17. None

Significance to HEART Research: Reduction of glomerular filtration rate and abnormalities of excretion of water and sodium are fairly constant features of cardiac failure, and elucidation of the mechanisms concerned should provide background information of great usefulness in the treatment of this disorder.

Proposed Course of Project: The studies at low rates of solute excretion are being extended.

The effect of substituting sodium salts for mannitol will be investigated.

The technique will be applied to the study of the effect of filtration rate changes on other discrete renal functions.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Kidney & Electrolyte Metabolism — LABORATORY OR BRANCH
3. _____ — SECTION
4. _____ — LOCATION(IF NOT BETH.)
5. NHI-17 — SERIAL NO.

6. The Mechanism of Water and Electrolyte Excretion in Nephrogenic Diabetes Insipidus — PROJECT TITLE

7. Dr. Jack Orloff — PRINCIPAL INVESTIGATOR(S)

8. Dr. Mackenzie Walser, Dr. Hans Keitel, Agnes Preston — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: An investigation of water and electrolyte excretion in patients with nephrogenic diabetes insipidus.

Objectives: It is the purpose of this project to attempt an elucidation of the mechanism of formation of dilute urines.

Methods Employed: Standard clearance techniques.

Patient Material: (1955 Calendar Year)

		No.	Average Stay- Days
Admissions:	Children, male	2	100
	Children, female	1	200
Outpatient:	Number of patients	0	

Major Findings: The free water clearance has been determined under a variety of conditions. In general, the data have been interpreted to indicate that urinary dilution is accomplished by three simultaneous processes: (1) abstraction of sodium and chloride, (2) passive diffusion of some water back to the plasma and (3) osmotic restriction of the rate of passive water diffusion by non-absorbed solute. This is in contrast to present hypothesis since it implies that even in the absence of pitressin the diluting segment is permeable to water.

Significance to Heart Research: These studies should provide information pertinent to the mechanism of excretion of water and sodium in normal man and lead to a better understanding of defects in cardiac failure.

Proposed Course of Project: These studies are to be extended to an investigation of similar processes in normal man and in disease states.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-17
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-17
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Kidney & Electrolyte Metabolism (LABORATORY OR BRANCH)

3. _____ (SECTION) 4. _____ (LOCATION (If NOT BETHESDA)) 5. NHI-18 (SERIAL NO.)

6. Renal Function in the Chicken
PROJECT TITLE

7. Dr. Jack Orloff
PRINCIPAL INVESTIGATOR(S)

8. Dr. Douglas Davidson and Doris Woods
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Investigation of mechanisms of electrolyte excretion in the chicken.

Objectives: By virtue of the unique anatomic arrangement in the chicken, it is possible to further elucidate the mechanisms involved in electrolyte transport in the renal tubule.

Methods Employed: Various solutions have been administered into one leg vein, urine has been collected from the kidney on that side and compared with urine from the contralateral kidney.

Major Findings: The major findings have been the following:

1. Alterations in CO_2 tension affect K excretion in a reciprocal manner. Thus, competition between hydrogen and potassium ions has been demonstrated.

2. Infusion of potassium salts at high rates results in saturation of potassium secretory mechanism.

3. Mercurial diuretics completely inhibit K secretion when it is initially low.

4. Ammonium salts when injected into one renal vein produce unilateral alkalinization of the urine and chloruresis.

5. Small doses of a carbonic anhydrase inhibitor produce unilateral effects, thus indicating that

the inhibitor need not be filtered to exert its effect.

Significance to Heart Research: It is hoped that these observations will help clarify the mechanisms of electrolyte transport in the kidney and be applicable to mammalian physiology.

Proposed Course of Project: These studies will be extended to investigate the chloruretic effect of ammonium salts.

1 c

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-18
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

16. None

17. None

Analysis of NIH Program Activities

Project Description

1. National Heart Institute
INSTITUTE

2. Kidney & Electrolyte Metabolism
LABORATORY OR BRANCH

3. None
SECTION

4. None
LOCATION (IF NOT BETHESDA)

5. NHI-19
SERIAL NO.

6. Changes in Renal Arterio-Venous Ammonia and glutamine Concentrations
PROJECT TITLE

7. Dr. Jack Orloff
PRINCIPAL INVESTIGATOR (S)

8. Dr. Douglas Davidson and Doris Woods
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION:

Project: To investigate effects of alderations in acid-base balance and urine pH on renal artery and vein ammonia and glutamine and the excretion of these substances.

Objectives: To elucidate some of the mechanisms wherby ammonia excretion is modified by urine pH and acid-base balance.

Methods Employed: Simultaneous femoral artery, renal vein and urinary ammonia and glutamine concentrations will be measured during infusions of various substances. Renal blood flow and glomerular filtration rate will also be measured.

Major Findings: A modification of a standard procedure for the measurement of blood ammonia has been perfected. Renal vein ammonia exceeds femoral artery ammonia in metabolic acidosis.

Significance to Heart Research: It is hoped to clarify the nature of the process of ammonia production and excretion as it is affected by metabolic alkalosis and acidosis. This is of importance with regard to acid-base metabolism in health and disease.

Proposed Course of Project: These studies have only recently been initiated and will be extended.

Analysis of NIH Program Activities

Budget Data

10. NHI-19
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications

15. NHI-19
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

The Mechanism of Ammonia Excretion in the Dog. Orloff, Jack and Berliner, Robert W. J.C.I., 1955 (accepted for publication).

17. None

Analysis of NIH Program Activities

Project Description

1. National Heart Institute — INSTITUTE
2. Kidney & Electrolyte Metabolism — LABORATORY OR BRANCH
3. _____ — SECTION
4. _____ — LOCATION(IF NOT BETHESDA)
5. NHI-20 — SERIAL NO.
6. The effect of Prednisone on Water and Electrolyte Excretion in Nephrosis — PROJECT TITLE
7. Dr. Jack Orloff and Dr. Mackenzie Walser — PRINCIPAL INVESTIGATOR(S)
8. Agnes Preston, Dr. James Baxter and Dr. Howard Goodman — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION

Objectives: To investigate the effect of steroid therapy in patients with the nephrotic syndrome on renal function and water and electrolyte excretion.

Methods Employed: Standard clearance techniques before, during and after therapy with Prednisone. Response to solute loading and water ingestion is also being studied.

Patient Material:

Patients reported elsewhere under the care of Dr. James Baxter are being used.

Major Findings: No predictable relationship has been noted between the initial level of glomerular filtration rate and the response to therapy with Prednisone. It appears that in responsive patients filtration rate increases during Prednisone administration and is maintained during remission. Late follow-up studies have not been performed. Changes in plasma volume have not been significant.

Significance to Heart Research: It is hoped to clarify the mechanism of Prednisone diuresis in patients with nephrosis. Furthermore, the relationship between filtration rate, water and solute excretion, and plasma volume is being investigated in an effort to further an understanding of the pathophysiology of this disease.

Proposed Course of Project: These studies are to be extended to a larger number of patients.

Analysis of NIH Program Activities

Budget Data

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications

15. NHI-20
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

The Effect of Prednisone on Water and Electrolyte Excretion. Schering Conference, New York, 1955. Jack Orloff.

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Kidney and Electrolyte Metabolism
LABORATORY OR BRANCH

5. NHI-21
SERIAL NO.

6. Anion Transport Across the Gastric Mucosa
PROJECT TITLE

7. Dr. C. Adrian M. Hogben
PRINCIPAL INVESTIGATOR(S)

8. Nordica Green
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To elucidate the dynamics of anion transport and to determine the nature of the anion transport system.

Methods Employed: The methods used in general have followed the principles set forth by Ussing, i.e., the measurement of unidirectional ion fluxes across isolated membranes in relation to the electrical potential with regulation of the electrical potential at desired levels.

Major Findings: Movement of two anions, chloride and thiocyanate, into and out of the epithelial layer of the frog gastric mucosa was measured, using radioinulin to characterize the two extracellular spaces of the mucosa. Transfer of chloride or thiocyanate across the secretory surface of the epithelial layer is ten times or more rapid than across the nutrient surface. The intracellular concentration of either anion is 2/3 that of the bathing solution and appears to be dependent on the electrical potential across the mucosa. Intracellular chloride exchanges completely within one hour.

The intracellular potassium concentration approximates the external sodium concentration while intracellular sodium is about 1/20 its external concentration. Potassium is actively transported from the nutrient to secretory surface and its exchange is more rapid across the nutrient surface of the epithelial cell layer than across the secretory surface. While sodium moves predominantly by passive diffusion about 100% of its movement occurs by active transport from the secretory to nutrient surface. The estimated electrical resistance of the gastric mucosa was found to be constant over a wide range of current density. Thiocyanate though markedly inhibiting hydrogen ion secretion at a high concentration of 25 mM/l does not inhibit beyond 90%. When the gastric mucosa is bathed by a salt solution containing nitrate ion instead of chloride, acid secretion is inhibited by 75%.

Significance to HEART Research: The frog gastric mucosa presents a more or less isolated anion transport system which can serve as a guide and model for anion transport systems elsewhere in the body.

Proposed Course of Project: Observations on the carbonic acid bicarbonate system in relation to chloride transport will be extended. Study of the flux of nitrate and its relationship to the movement of chloride will be carried out.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-21
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-21
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Hogben, C. A. M.: Active transport of chloride by isolated frog gastric epithelium: Origin of the gastric mucosal potential. Am. J. Physiol., 180:641-649, March 1955.

Hogben, C. A. M.: Biological aspects of active chloride transport. In "Electrolytes in Biological Systems." Editor Shanes, A. Am. Physiol. Soc., Washington, D.C., 1955.

Hogben, C. A. M.: The mechanism of gastric acid secretion as revealed by radioisotopes. U. N. Conference on Peaceful Uses of Atomic Energy. 1955.

Hogben, C. A. M.: Gastric secretion of chloride and weak electrolytes. Comments in Symposium on Active Transport of Weak Electrolytes. University of Wisconsin (In Press)

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Kidney and Electrolyte Metabolism
LABORATORY OR BRANCH

5. NHI-22
SERIAL NO.

6. Gastrointestinal Absorption and Secretion of Drugs
PROJECT TITLE

7. Dr. C. Adrian M. Hogben
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The rates of movement of diverse compounds across the gastrointestinal tract will permit characterization of membrane permeability and significant physical chemical factors.

Methods Employed: A saline solution containing a drug is perfused through rat intestine in situ. The decreased concentration of the drug leaving the intestine provides a measure of absorption and an apparent permeability coefficient.

Major Findings: Most of the weak acids, aniline and deuterium oxide, are absorbed rapidly at a limiting rate of 50%. The heterogeneity of compounds exhibiting this absorption limit suggests that a factor other than the epithelial surface regulates absorption, either blood flow or radial diffusion within the lumen. Bases other than aniline are absorbed more slowly, the stronger the base the slower the absorption.

Significance to HEART Research: This study will provide for the first time a quantitative picture of gastrointestinal absorption of drugs. Analysis of factors governing weak electrolyte transfer will contribute information to the study of electrolyte exchange across the gut. Investigation of factors other than permeability are significant for other aspects of gastrointestinal physiology such as fat absorption.

Proposed Course of Project: The limiting rate of intestinal absorption will be studied in terms of plasma binding, visceral blood flow and mixing within the intestinal lumen. Rates of absorption will be determined as a function of concentration and pH.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-22
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Dr. Lewis S. Schanker and Dr. Parkhurst A. Shore,
Laboratory of Chemical Pharmacology, NHI

14. None

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-22
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Kidney and Electrolyte Metabolism
LABORATORY OR BRANCH

5. NHI-23C
SERIAL NO.

6. Studies of Hyponatremia
PROJECT TITLE

7. Dr. Mackenzie Walser and Dr. Jack Orloff
PRINCIPAL INVESTIGATOR(S)

8. Agnes Preston and Raymond Cherwinski
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To clarify the mechanism of development of plasma hypo-osmolality.

Methods Employed: Standard clearance and balance techniques.

Patient Material: (1955 Calendar Year)

Admissions: None specifically for this study. Appropriate patients of other investigators have been studied.

Major Findings: The study has indicated that in three patients studied, inadequate water excretion, and not sodium depletion, was the cause of reduced plasma tonicity. Despite this, free water clearance following a water load was apparently normal in two, indicating no basic defect in the urinary diluting mechanism.

Significance to HEART Research: It is hoped that these studies will provide information concerning the mechanism and control of hyponatremia. This is an especially important clinical problem since the condition is noted frequently in edematous patients.

Proposed Course of Project: These studies are to be extended to an investigation of hyponatremia in patients with renal, cardiac, liver and other diseases.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-23C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards and Publications

15. NHI-23C
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Kidney and Electrolyte Metabolism
LABORATORY OR BRANCH

5. NHI-24
SERIAL NO.

6. Measurement of Gastric Blood Flow
PROJECT TITLE

7. Dr. C. Adrian M. Hogben
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To devise a method for measuring blood flow to the gastric mucosa in the intact individual.

Methods Employed: The principle of the method is based on the observation that the rate of appearance of certain basic chemical compounds in the gastric juice appears to be limited by the blood flow. This suggests the possibility that the measurement of the rate of appearance in gastric juice in relation to the concentration in the blood might serve as a measure of the blood flow. Studies have been done on dogs with Heidenhain pouches and other acute experiments on operated dogs.

Major Findings: The test material previously used, neutral red, was abandoned because of low sensitivity of plasma determination and large systemic arterio-venous differences. The base quinine was cleared by the dog gastric pouch more rapidly at higher plasma concentrations. This can be attributed to the strong plasma binding of quinine which makes quinine an unsuitable test substance. The in vitro movement of quinine is much greater from serosa to mucosa than in the

opposite direction. The mechanism of base secretion was tested using 5 mM/l of thiocyanate to inhibit acid secretion and highly buffered media. In spite of these measures to reduce the pH gradient in vitro, quinine continued to move more rapidly from serosa to mucosa.

Significance to HEART Research: This project represents the application to a practical purpose of an observation in a totally unrelated field, namely a study of the disposition of drugs in the body.

Proposed Course of Project: The base aniline will be used for further in vivo studies because it is not bound to plasma proteins. A comparison will be made between aniline clearance and radiosodium uptake by the gastric mucosa. In vitro investigation of the mechanism of base secretion will be pursued with higher concentrations of thiocyanate and buffer.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-24
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards and Publications

15. NHI-24
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Kidney and Electrolyte Metabolism and Cardiovascular Hemodynamics
LABORATORY OR BRANCH

5. NHI-25C
SERIAL NO.

6. The Action of Human Plasma on the Isolated Frog Heart
PROJECT TITLE

7. Dr. Stephen Hajdu and Dr. Edward Leonard
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Observation of the action of human plasma on the isolated frog heart; comparison of subjects with and without essential hypertension.

Objectives: The plasma of patients with essential hypertension has an action on the isolated frog heart similar to that of certain known steroids, e.g. D.O.C., progesterone, and cardiac glycosides. The objective of the study was to compare the amounts of this activity in subjects with and without essential hypertension.

Methods Employed: Subjects for the study were divided into three groups: patients with essential hypertension, patients without essential hypertension (both normotensives and patients with other types of hypertension in this group) and normal controls. The activity of the plasma of each subject was measured, being compared to the action of a known concentration of strophanthidin.

Patient Material: (1955 Calendar Year)

Admissions: None specifically for this study. Appropriate patients of other investigators have been studied.

Major Findings: Thirteen of the 14 normal plasmas exhibited activity corresponding to less than 0.5 microgram of strophanthidin. Likewise, 13 of the 14 plasmas from patients without essential hypertension fell into the less than 0.5 microgram range. Thirteen of the 15 plasmas from patients with essential hypertension exhibited activity of more than 1.25 micrograms.

Significance to HEART Research: The cause of essential hypertension is unknown, and the diagnosis is one of exclusion – made only after known causes have been ruled out. The above findings may eventually prove useful as a diagnostic test in doubtful cases. Furthermore, there is a possibility that the difference demonstrated between normal and hypertensive plasma may be due to a substance which is important in the etiology of the disease.

Proposed Course of Project: It is planned to further investigate the nature of the plasma substance and to characterize it by comparing its action on bioassay systems (heart and blood vessel) to that of certain known substances. Comparison of essential hypertension plasma with that of hog plasma will be made, since if both species contain the same material hog plasma would be a good source for developing fractionation procedures.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-25C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications

15. NHI-25C
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Kidney and Electrolyte Metabolism
LABORATORY OR BRANCH

5. NHI-26C
SERIAL NO.

6. Kidney Function in Sickle Cell Anemia
PROJECT TITLE

7. Dr. Hans G. Keitel
PRINCIPAL INVESTIGATOR(S)

8. Dr. H. Goodman, Dr. J. Baxter and Dr. D. Thompson
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Kidney function in sickle cell anemia

Objectives: To evaluate possible factors which result in hyposthenuria, hematuria and renal failure in sickle cell anemia.

Methods Employed: (A) Tests of renal function:

1. Concentration and dilution maxima
2. Glomerular filtration rate
3. Quantitative urinary output of formed element

(B) Effect of suppression of sicklemia by replacement and (ordinary) red cell infusions on (A) above.

Patient Material: (1955 calendar year)

		No.	Average Stay - Days
Admissions:	Adults	2	60
Outpatient:	Number of patients	60	
	Number of visits	300	

Major Findings: 1. Hyposthenuria, w ich is found regularly in sickle cell anemia, was found in 70% of asymptomatic subjects with the sickle cell trait. Hyposthenuria was completely corrected by the administration of multiple red cell transfusions in four young sickle cell anemia patients but not in two young adults. The defect apparently is reversible and therefore not a congenital defect as postulated earlier.

2. Leucocytosis, which is found in most untreated sickle cell anemia patients, and sickle cell pains were repressed by small red cell exchange and ordinary transfusions. Evidence is accumulating that the leucocytosis is not a simple consequence of a hyperplastic bone marrow and hemolytic anemia (see attached sheet).

Significance to HEART Research: Provide information concerning the renal mechanisms involved in the clearance and conservation of water.

Proposed Course of Project: A. Concentration Defect: Normal and sickle cell hemoglobin are being administered intravenously (by Colonel Crosby at Walter Reed Army Medical Center) during hydropenia and pitressin administration to determine whether hemoglobinuria of normal or sickle cell hemoglobin is more deleterious to the renal concentration mechanism.

B. Study the maximal water clearance in further sickle cell anemia patients and in "trait" and normal subjects. Preliminary data in icate an enhanced urinary diluting ability (minimal urine osmolality and maximal minute urinary output) during sustained water diuresis in some sickle cell anemia patients.

C. Confirm existing evidence of the suppressive effect of small red cell transfusions (and replacement transfusions) on vascular occlusions.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-26C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Colonel Crosby, Walter Reed Army Medical Center

14. None

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-26C
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

The urinary concentration defect in sicklemia. Keitel, H. G. and Thompson, C. : Clin. Res. Proc. 4:7 (1956).

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Kidney and Electrolyte Metabolism
LABORATORY OR BRANCH

5. NHI-27
SERIAL NO.

6. Cation Transfer by Human Red Cells
PROJECT TITLE

7. Dr. Edward T. Dunham
PRINCIPAL INVESTIGATOR(S)

8. Mrs. Jean Johnson
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The ultimate objective of this project is an understanding of the process by which electrochemical gradients of sodium and potassium are maintained across cell membranes.

Methods Employed: Red cells from freshly drawn human blood are suspended in simulated plasma or modified media and maintained in suspension at constant temperature in a Barcroft-Warburg apparatus. By means of established and modified tracer procedures, unidirectional movements (fluxes) of ions across the cell membrane are followed and analyzed.

Major Findings: A marked influence of pH on both active (metabolically dependent) and passive transfer of these ions has been demonstrated. A close linkage between active sodium outflux and active potassium influx has been observed and the nature of this linkage has been examined. Improved equations for the analysis of two-compartment unsteady state kinetics have been developed.

Significance to HEART Research: The human erythrocyte provides one of the least complex and most easily manipulated systems for an intensive study of active cation transport across cell membranes. An understanding of the mechanism of active cation transport will further our appreciation of the cellular regulation of electrolyte metabolism.

Proposed Course of Project: Current research will be extended. An investigation of the relationship between exergonic enzymatic reactions and metabolically linked cation fluxes will be pursued. In this regard, immediate attention will be given to the role of ATP hydrolysis.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-27
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications

15. NHI-27
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Kidney and Electrolyte Metabolism
LABORATORY OR BRANCH

5. NHI-28
SERIAL NO.

6. C^{14}-Labelled Inulin as a Tracer for Inulin
PROJECT TITLE

7. Dr. Ernest Cotlove
PRINCIPAL INVESTIGATOR(S)

8. Harry Morrison
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Evaluation of inulin C^{14} carboxylate as a tracer for inulin and application to measurement of renal function and extracellular fluid.

Objectives: The object of this project is to develop a tracer for inulin which will 1) simplify the analytical procedure for the measurement of inulin clearance, 2) permit the analysis of inulin at very low concentration in body fluid and tissue by elimination of blanks which interfere with the colorimetric methods, and 3) enable study of kinetics of distribution and excretion.

Methods Employed: The physiologic procedures which have been employed are standard procedures for the evaluation of renal function. Varying degrees of equilibration of inulin in the body are obtained with a constant infusion and the post-infusion rate of excretion is determined.

Major Findings: The renal clearance of inulin carboxylate appears to exceed that of non-labelled inulin by a few percent, explicable in terms of Donnan distribution, but further data are needed to clarify this point. The measurement of the post-infusion rate of excretion of radioactivity is giving

in lication, in intact humans and animals, of rapidly and slowly exchanging volumes of extracellular fluid as was indicated by direct tissue analysis in animals. The method of infinite thickness liquid counting of C-14 has been adapted to give a ten-fold increase in counting rate, with consequent increased accuracy and sensitivity. Radioactive inulin has been used in other projects for determining extracellular volumes of tissues in vitro.

Significance to HEART Research: The availability of C-14 inulin for the measurement of glomerular filtration together with the simple and accurate method of radioassay which has been developed should greatly simplify and possibly increase the accuracy of this procedure for evaluation of kidney function. Measurement of extracellular volume and of the rates of exchange of various portions of extracellular fluid should provide basic information in understanding the physiologic control of extracellular volume and the abnormal collections of fluid in edematous states (as in heart failure, nephrosis, cirrhosis of liver).

Proposed Course of Project: Further studies will be done using C-14 inulin in normal human subjects and in patients on the comparative clearances of radioactive inulin; on the volumes and rates of exchange of extracellular fluid; and on the correlation of extracellular volume and the amount of secretion of the salt-conserving hormone aldosterone. Further studies of C-14 inulin distribution in individual tissues of animals will also be done.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-28
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards and Publications

15. NHI-28
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Kidney and Electrolyte Metabolism
LABORATORY OR BRANCH

5. NHI-29
SERIAL NO.

6. Method and Apparatus for Automatic Titration of Chloride
PROJECT TITLE

7. Dr. Ernest Cotlove
PRINCIPAL INVESTIGATOR(S)

8. Harry Morrison
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Development of method and apparatus for rapid, precise and automatic titration of chloride.

Objectives: The object of this project is to develop a simple, rapid, precise and sensitive method that will permit a detailed investigation of tissue chloride contents, cellular chloride content and exchangeability of chloride.

Methods Employed: The analytic method is based on titration of chloride by silver ion. The end-point is detected amperometrically by the sudden rise in diffusion current at a rotating platinum electrode, due to free silver ion. The curve of the diffusion current is plotted on paper by a Varian graphic recorder. The silver ion is delivered at a constant rate by electrolysis of metallic silver with constant current. The distance on the paper is proportional to the titration value of silver ion. The procedure is automatic.

Major Findings: A method and simplified apparatus has been developed which provides a high degree of precision, is very rapid, is suitable for small amounts of tissue chloride, and can be performed by relatively untrained personnel.

Significance to HEART Research: A simple, rapid and accurate method for determination of chloride in biological materials will be of value, since chloride is the predominant anion in extracellular fluid, and current methods are slow and depend on operator skill and judgment. The analysis of chloride in tissues in particular has presented problems. The present method should be of great usefulness not only in specialized research problems but also in clinical laboratories involved in patient care.

Proposed Course of Project: The method will be applied to the problems for which it was developed, namely a study of tissue chloride. A somewhat simpler and less expensive modification of the present apparatus will be developed and tested for applications (particularly clinical) which require much less sensitivity and possibly somewhat less precision.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-29
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

This project is being conducted in collaboration with Dr. Robert L. Bowman and Mr. Hillary Trantham, Laboratory of Technical Development, NHI

14. None

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-29
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Kidney and Electrolyte Metabolism
LABORATORY OR BRANCH

5. NHI-29(1)
SERIAL NO.

6. Ionic Exchange in Secreting Cells
PROJECT TITLE

7. Dr. Ernest Cotlove and Dr. C. Adrian M. Hogben
PRINCIPAL INVESTIGATOR(S)

8. Harry Morrison; Nordica Green; Shendrine B. Henry
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Study of ionic exchange in secreting cells.

Objectives: The object of this project is to study ionic exchange in secreting cells, particularly any differences in exchange rates at the opposite nutrient and secretory surfaces, and the effects of experimental variables.

Methods Employed: The isolated frog gastric mucosa is mounted between lucite chambers and bathed by modified Ringer's solution on the nutrient and secretory surfaces. The electrical potential and current between the solutions is measured and any desired potential can be imposed. Radioactive or stable ions are introduced into or analyzed in samples from the bathing solution. The mucosa is analyzed by standard methods for tissue analysis. The extracellular spaces are determined with C-14 inulin.

Major Findings: The major finding of this project during the past year has been a marked asymmetry of exchange rates of radioactive chloride (Cl-36) at the opposite poles of the frog gastric epithelial cells, the exchange at the secretory surface being up to ten times as fast as at the nutrient surface. A similar asymmetry was noted with thiocyanate ($S^{35}CN$) ion. Both anions

have been shown by Dr. Hogben to be actively transported across the epithelium from nutrient to secretory bathing solution. If equal concentrations of radioactivity are introduced into the bathing solutions on both surfaces all of the cellular chloride (which is high in gastric mucosal cells) is exchangeable.

Significance to HEART Research: Information on the basic processes by which cells maintain their composition and perform their activities is essential to an understanding of the function of the organs, such as heart contraction, gastric secretion or renal excretion. The present study contributes information on the mechanism and possible localization of chloride secretion, and to interpretation of overall exchange rates studied in intact animals and humans.

Proposed Course of Project: Further studies will be done on bi-facial chloride exchange and the effects of composition of the bathing solutions, imposed potential and metabolic state on the asymmetrical exchange rates. The exchange of other ions and of water tracers will be studied.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-29(1)
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications

15. NHI-29(1)
SERIAL NO.

16. None

17. None

1. a

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Cellular Physiology and Metabolism
LABORATORY OR BRANCH

3.
SECTION

4.
LOCATION (IF OTHER THAN BETHESDA)

5. NHI-30
SERIAL NO

6. Electron Transport Phosphorylation
PROJECT TITLE

7. W. Wayne Kielley
PRINCIPAL INVESTIGATOR(S)

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Electron transport phosphorylation

Objectives: To identify the mechanism by which "high energy phosphate bonds" are formed in association with electron flux over the terminal electron transport system.

Methods Employed: Enzymatically active materials are prepared from liver by standard procedures of centrifugal fractionation of intracellular components. Bacterial material is obtained from large scale culturing of the desired organism. These materials (liver mitochondria and bacteria) are subjected to a variety of physical and chemical technics designed to produce smaller particles or soluble enzymes. Standard technics of studying the enzymatic activity of these preparations are employed. Radioactive phosphate is routinely employed for phosphorylation studies coupled with chromatographic methods of analysis.

Major Findings: Identification of the mechanism of electron transport phosphorylation has so far been hampered by the inability to recognize the process in any system other than intact mitochondria. Three stages of phosphorylation are known to occur in the oxidation of reduced diphosphopyridine nucleotide (DPNH) by oxygen (two in the case of succinate) so that not one but three mechanisms of phosphate esterification are operating in these particles. In an attempt to investigate a one-stage phosphorylation process we have examined two limited systems: (1) The pyruvic oxidase of L. delbrueckii and (2) the coupling of DPNH oxidation to the reduction of crotonyl coenzyme A in Cl. kluyveri. Both systems involve flavoproteins. The first one was regarded as a model for a possible "high energy" reduced flavin intermediate. The second system, which is presumed to be the major source of "high energy" phosphate for this organism (C. kluyveri) offers the particular advantages of phosphorylation only at the flavoprotein level since no iron porphyrin pigments are present in the organism.

1. L. delbrueckii pyruvic oxidase. The enzyme was prepared from dried cells of the organism and purified about 100-fold. The hypothesis presented in a previous report was tested using substrate levels of the enzyme in the spectrophotometer. In this hypothesis, the conversion of pyruvate to acetyl phosphate and CO_2 would involve as an intermediate reduced, acetylated flavine-adenine dinucleotide. Phosphorolysis of this would then yield acetyl phosphate. If the mechanism were correct then reduction of the enzyme flavin should occur in the presence of pyruvate and absence of phosphate. Since it was not possible to observe reduction of the flavin under these conditions, it must be concluded that phosphate participates in the reaction prior to involvement of the flavin and the latter must serve as a simple hydrogen or electron acceptor.

2. Phosphorylation in the coupling of DPNH to crotonate reduction in C. kluyveri. Although the couple

DPNH → DPN
crotonyl-SR → butyryl SR

proceeds with a free energy change of about 23,000 cal.--sufficient for formation of two high-energy phosphate bonds--and the coupled reaction can readily be observed with cell free extracts, it has been impossible so far to detect any phosphorylation dependent on the electron transfer process and that cannot be identified as a "substrate level" reaction. It is concluded that if the proposed mechanism is actually the energy source for this organism, currently available means of preparing bacterial extracts are incapable of producing active preparations.

Significance to HEART Research: In the complete oxidation of a glucose molecule by an aerobic tissue such as heart muscle, it is estimated that some 36 or 38 high energy phosphate bonds (net) are formed through glycolysis and the tricarboxylic acid cycle. Of the total only two arise from glycolysis and only four are identified as "substrate level phosphorylation," i. e. they arise from a direct reaction of the substrate with phosphate or through phosphorylysis of a bond involving the substrate. The other 32 or 34 high energy phosphate bonds arise by the process we identify as electron transport phosphorylation or, less correctly, as oxidative phosphorylation. The mechanism of the process of electron transport phosphorylation is completely unknown. The importance of this process in the energy relationships of aerobic cells is evident.

Proposed Course of Project: electron transport phosphorylation: Recently introduced procedures for disintegrating bacteria and intracellular particles will be examined for their effectiveness in producing actively phosphorylating extracts of C. kluyveri and liver mitochondria. It is planned to again examine the reciprocal relationship between phosphorylating potential and adenosine triphosphatase activity in these systems.

1. c

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-30
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-30
SERIAL NO.

16. NONE
PUBLICATIONS

17. Travel Award (American Society of Biological Chemists - through National Science Foundation) to attend 3rd International Congress of Biochemistry, Brussels, July, 1955.

2. a

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Cellular Physiology and Metabolism
LABORATORY OR BRANCH

3. _____
SECTION

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. NHI-31
SERIAL NO

6. Enzymatic Degradation of the Cholesterol Side Chain
PROJECT TITLE

7. Marjorie G. Horning
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Enzymatic Degradation of the Cholesterol Side Chain

Objectives: The immediate objective of this research is to isolate and determine the chemical nature of unidentified co-factor(s) shown to be required for the oxidation of the side chain cholesterol to CO_2 by particulate enzyme systems obtained from liver.

Methods Employed: A reproducible method of isolation of the cofactor from mouse liver has been developed which involves charcoal, resin and paper chromatography. This procdure has not been found applicable to large scale isolation (calf liver) due primarily to the instability of the cofactor during the initial steps of isolation.

Microchemical analyses of the purified fraction obtained from mouse liver have provided considerable information on the chemical nature of the cofactor.

Since the chemical analyses indicated the presence of organic phosphate, a barium fractionation procedure was tried. This method gave a very low yield of very active material, and provides an additional means of studying this problem.

Major Findings: The major finding of this project has been the isolation of an enzymatically active fraction which gave only one ninhydrin positive spot on paper chromatography and upon analysis was found to contain organic phosphate, a reducing group, α-amino nitrogen and a base in a molar ratio of 1 for all components. This purified fraction can be hydrolyzed enzymatically with phosphatase thus permitting analysis for the unaltered components. The conditions for complete hydrolysis of the organic phosphate chemically are so severe that it is possible that partial destruction and chemical transformation of the other constituents has occurred.

Significance to HEART Research: Although cholesterol has been implicated as a causative agent in the etiology of atherosclerosis, its exact role is not known. It is possible that the deposition of cholesterol in the plaques is due to the inability of the body to dispose of this sterol through normal metabolic processes. The experiments described in this report should provide information about the cofactors required for cholesterol metabolism. After identification, these cofactors could be evaluated as dietary supplements in regulating cholesterol metabolism in humans.

Proposed Course of Project: The research work will be concerned with the characterization of the cofactor by microchemical and enzymatic analysis. This approach has provided so much valuable information in recent months that it will be emphasized. Since these microchemical analyses have made possible a partial characterization of the cofactor the problem of isolation on a larger scale coupled with barium fractionation will be re-examined. It is possible that this cofactor is not specific for the degradation of the side chain of cholesterol, but is a general cofactor required for the oxidative degradation of branched chain or isoprenoid units; many compounds of this type occur naturally and are physiologically active. Using the enrichment culture technique, it may be possible to isolate a microorganism with the enzymes to degrade this type structure and thus study the enzymatic aspect of side chain degradation.

2. c

Analysis of NHI Program Activities

Budget Data Sheet

10. NHI-31
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-31
SERIAL NO.

16. NONE
PUBLICATIONS

17. NONE
HONORS AND AWARDS

3. a Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Laboratory of Cellular Physiology and Metabolism — LABORATORY OR BRANCH
3. _____ — SECTION
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-32 — SERIAL NO.

6. The Enzymatic Synthesis of Energy-rich Compounds and Their Utilization in Biosynthetic Reactions. — PROJECT TITLE

7. Dr. E. R. Stadtman — PRINCIPAL INVESTIGATOR

8. Dr. I. Harary — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: The enzymatic synthesis of energy-rich compounds and their utilization in biosynthetic reactions.

Objectives: It is the object of this project to study those enzymatic reactions which lead to the formation of substances with high free energies of hydrolysis and to determine how the potential energy of such compounds is used to promote various biosynthetic reactions.

Methods Employed: By means of the enrichment culture technique, microorganisms are selected whose metabolism is specifically concerned with the dissimilation of substances of particular biochemical interest. Cell-free extracts and purified enzyme preparations of these organisms are used to elucidate the biochemical mechanism of the metabolism of the various compounds. These studies are supplemented with similar studies using tissue extracts of animals.

Major Findings: A new strain of the microorganism Clostridium propionicum has been isolated from soil enrichment cultures and was shown to catalyze the fermentation of α-alanine, β-alanine, lactate, acrylate, serine and pyruvate to mixtures of acetate, propionate, carbon dioxide and ammonia. Studies with enzyme preparations have shown that propionate oxidation by this organism proceeds by way of acyl-CoA derivatives. Two heretofore unrecognized thiol ester compounds have been established as intermediates in this oxidation; namely, acrylyl-CoA and β-alanyl CoA.

Dr. I. Harary has succeeded in isolating an organism from the soil which catalyzes the decomposition of nicotinic acid to one mole each of acetate, propionate, carbon dioxide and ammonia. Preliminary results with enzyme preparations indicate that 5-hydroxy-nicotinic acid is the first intermediary in this conversion.

Experiments carried out in collaboration with Professor Lynen (Munich, Germany) demonstrated that an enzyme system is present in cell-free extract of C. kluyveri that catalyzes the reduction of crotonyl CoA to butyryl CoA utilizing reduced diphosphopyridine

nucleotide as the electron donor. This reaction is of particular interest since it can theoretically provide sufficient energy for two energy-rich phosphate bonds and it represents the only obvious mechanism by which C. kluyveri can obtain energy for growth. It therefore represents an ideal model system for the study of oxidative phosphorylation at the pyridine nucleotide-flavin level.

Microorganisms which catalyze the oxidation of lumichrome to CO_2 and ammonia have been isolated from soil enrichment cultures.

Significance to HEART Research: This project is part of the basic research program of the Heart Institute and although there is no immediate obvious relationship to heart disease, this research will result in the accumulation of fundamental information on one of the more important aspects of intermediary metabolism; namely, that concerned with the biochemical mechanism of deriving chemical energy from food materials and the utilization of this energy for the maintenance and synthesis of cellular constituents. Such information will undoubtedly lead eventually to a better understanding of heart metabolism and degenerative diseases.

Proposed Course of Project: The above studies will be continued. In particular it is planned to investigate the further metabolism of Acrylyl CoA and β-alanyl CoA and to initiate studies on the mechanism of lumichrome oxidation by the lumichrome oxidizing bacterium. An effort will be made to isolate microorganisms that can oxidize tocopherol and Vitamin K. Such microorganisms should serve as excellent model systems to study the mechanism of oxidation of compounds with phytyl side chains (such as cholesterol). Studies on the role of imidazole acylation in energy metabolism are also planned.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-32
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-32
SERIAL NO.

16. Fermentations De L'Acide Propionique, Bull. Ste'. Chim. Biol. 37, Numbers 9 and 10, 931 (1955).

The enzymatic Synthesis of β-alanyl CoA, J. Am. Chem. Soc. 77, 5765 (1955).

Isolation and Characterization of 6,7 Dimethyl-9-(2'-hydroxyethyl) isoalloxazine as a bacterial Fermentation Product of Riboflavin. (With H. T. Miles) J. Am. Chem. Soc. (1955).

Aldehyde Dehydrogenase from Clostridium kluyveri (with R. M. Burton). Methods in Enzymology I, 518 (1955).

Coenzyme Transphorase from Clostridium kluyveri (with H. A. Barker, and A. Kornberg) ibid., 599 (1955).

Phosphotransacetylase from Clostridium kluyveri ibid., 596, (1955).

In Press:

Bacterial Degradation of Nicotinic Acid (by I. Harary). Nature (1956).

The Preparation and Assay of Acetyl Phosphate. Methods in Enzymology III, (1956).

Enzymatic Thiotransacetylation (with R. C. Brady) - Current Research in Neurological Chemistry (book)

The Preparation of Coenzyme A (with A. Kornberg) Methods in Enzymology.

The Preparation and Assay of Acyl Coenzyme A and Other Thioesters; Use of Hydroxylamine.

17. Awarded an honorary medallion by the Societe' De Chimie Biologique, Paris, France. June, 1955.

4. a Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Cellular Physiology and Metabolism
LABORATORY OR BRANCH

3. _____
SECTION

4. _____
LOCATION(IF OTHER THAN BETHESDA)

5. NHI-33
SERIAL NO.

6. Studies on Heparin Metabolism
PROJECT TITLE

7. Dr. Edward D. Korn
PRINCIPAL INVESTIGATOR

8. Dr. A. Nail Payza
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Studies on Heparin Metabolism

Objectives: To study the enzymic mechanism of heparin degradation and synthesis. To elucidate the structure of heparin through the identification of intermediates obtained from enzymic hydrolysis. To study the possible biological activity of partial degradation products of heparin.

Methods Employed: A Flavobacterium which was isolated previously is grown on large scale and then adapted to heparin utilization. Acetone-dried preparations of the bacteria are prepared and extracted with dilute buffer. The extracts are incubated with heparin and the degradation products analyzed.

Major Findings: Procedures for the growth, adaptation and extraction of the bacteria have been developed. The mixture of products from the digestion of heparin by the bacterial extracts have been analyzed and preliminary steps taken toward the separation in pure form of the several products. The general characteristics of these compounds has already been determined.

Significance to HEART Research: Heparin is employed clinically as an anticoagulant. It also has the ability to induce rapid clearing of blood lipemia through the release of lipoprotein lipase with which it appears to be associated. In order to understand better the mechanism of heparin action, it is first necessary to know the structure of the compound. Analysis of the enzymatic degradation products of heparin should permit this. Also it may be possible to obtain a less complicated molecule which can be synthesized in the laboratory that would have the desired properties of heparin.

Proposed Course of Project: It is hoped to be able to isolate and identify the products of heparin degradation and to further define the properties of the enzymes involved.

4. b Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-33
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. Foreign Operation Administration Exchange Visitor-Scientist
Dr. A. Nail Payza.

14. NONE

Analysis of NIH Program Activities
Honors, Awards, and Publications Sheet

15. NHI-33
SERIAL NO.

16. Bacterial Degradation of Heparin, A. Nail Payza and E. D. Korn, Nature, January 1956.

17. NONE
HONORS AND AWARDS

5. a Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Laboratory of Cellular Physiology and Metabolism — LABORATORY OR BRANCH
3. _____ — SECTION
4. _____ — LOCATION(IF OTHER THAN BETHESDA)
5. NHI-34 — SERIAL NO

6. Studies on Lipoprotein Lipase (Clearing Factor) — PROJECT TITLE

7. Dr. Edward D. Korn — PRINCIPAL INVESTIGATOR

8. Mr. Thomas W. Quigley, Jr. — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Studies on Lipoprotein Lipase (Clearing Factor)

Objectives: To study the properties of this enzyme in relation to lipoprotein and fat metabolism. To clarify the possible role of heparin in reactions catalyzed by lipoprotein lipase.

Methods Employed: Chicken adipose tissue is defatted with acetone and the residual material extracted with dilute ammonia. This extract contains the enzyme in a relatively high state of purity.

Major Findings: It was found that chicken fat provides a rich and readily available source of lipoprotein lipase. A procedure has been developed by which the fat is extracted with room temperature acetone and the residual material then extracted with 0.025 M NH_3. This extract is then lyophilized and the resultant powder is quite stable and soluble in water. From 5 lbs. of fat approximately 4 g. of lyophilized powder are obtained. It has been demonstrated that this enzyme is identical to that obtained from heart and post-heparin plasma. This material is the most active that we have ever had. Preliminary attempts at further purification have been begun.

Significance to HEART Research: Abnormal lipoprotein transport or metabolism appears to be associated with the incidence of atherosclerosis. Lipoprotein lipase is the only known enzyme system for the degradation of these compounds. Therefore, a study of its properties should greatly increase our knowledge of the manner in which fat is handled by the body.

Proposed Course of Project: Further attempts at purification of the chicken fat enzyme will be made. Experiments are also under way to determine the effect of the bacterial heparinase on lipoprotein lipase activity in order to determine if heparin is an enzyme-bound co-factor for the lipase.

5 b

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-34
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-34
SERIAL NO.

16. Studies on lipoprotein lipase of rat heart and adipose tissue. Edward D. Korn and Thomas W. Quigley, Biochimica et Biophysica Acta 18, 143 (1955).

Clearing factor, a heparin-activated lipoprotein lipase. I. Isolation and characterization of the enzyme from normal rat heart, E. D. Korn, J. Biol. Chem. 215, 1 (1955).

Clearing factor, a heparin-activated lipoprotein lipase. II. Substrate specificity and activation of coconut oil, E. D. Korn, J. Biol. Chem. 215, 15 (1955).

17. NONE
HONORS AND AWARDS

6. a Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Laboratory of Cellular Physiology and Metabolism (LABORATORY OR BRANCH)

3. _____ (SECTION) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NHI-35 (SERIAL NO.)

6. 1. Enzymatic degradation of glycine, proline, serine and lysine.
2. The role of C_1 compounds in intermediary metabolism.
(PROJECT TITLE)

7. Thressa C. Stadtman
(PRINCIPAL INVESTIGATOR)

8. None
(OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Project: 1. Enzymatic degradation of glycine, proline, serine and lysine.
2. The role of C_1 compounds in intermediary metabolism.

Objectives: The objective of this research is to establish the biochemical pathways by which lysine, serine, proline, glycine and one-carbon compounds are metabolized.

Methods Employed: Cell-free extracts are prepared of an anaerobic bacterium, Clostridium HF, which has been shown to have a particularly active amino acid metabolism. Studies on the dissimilation of lysine, serine, proline and glycine by these cell-free preparations are carried out using the standard techniques of enzyme chemistry.

Major Findings: The enzyme systems that reductively degrade proline and glycine have been isolated and partially purified from extracts of Cl. HF, a member of the group of anaerobic bacteria that depend on such reactions for their energy needs. The discovery that reduced lipoic acid can serve as the electron donor for these reactions (and equally well a model compound-- 1,3-dithiol propanol) rendered the system much more amenable to detailed study and purification. Cofactor requirements for these reactions have been established and purification of the glycine system has yielded an enzyme fraction that causes the accumulation of an unidentified metabolite of glycine. The data suggest this may be closely related to or perhaps an actual intermediate in the conversion of glycine to acetic acid. Progress has been made in obtaining more active enzyme preparations from bacteria with special bacterial presses. Those from Cl. HF decompose lysine much more rapidly and also retain the ability to synthesize acetic acid from 2 one-carbon molecules--formic acid.

Significance to HEART Research: This is one of the basic research projects of the Heart Institute established for the purpose of securing fundamental information on the intermediary metabolism of amino acids and one-carbon compounds. Basic information on these metabolic processes is essential to the ultimate understanding of heart metabolism as well as metabolism in general.

Proposed Course of Project: Currently the isolation and identification of the metabolite of glycine mentioned above is being pursued. The overall aim of this aspect of the work is to establish as much as possible the nature of the intermediates and electron transport system involved in the reduction of proline to δ-amino valeric acid and glycine to acetate and ammonia. The nature of the naturally occurring sulfhydryl catalyst in the system is of particular interest.

With the better techniques now available for preparing extracts from tissues and bacteria it is feasible to examine in such preparations the interesting synthesis of $C^{14}H_3C^{14}OOH$ from 2 $HC^{14}OOH$ that can be shown to occur. Previously it was only possible to study this process in intact cells where intermediate steps could not be examined.

6. c

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-35
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. Support for research carried out in the laboratory of Dr. D. D. Woods, Univ. of Oxford, from Jan. to May, 1955 and Dr. F. Lynen, Univ. of München, from May to August, 1955 was obtained from the Helen Hay Whitney Foundation and administered by the U. S. Public Health Service as a Special Fellowship.

14. Some collaborative research on serine metabolism and its conversion to the methyl carbon of methionine was carried out at Oxford with another guest scientist, Dr. J. Szulmajster of Paris. Exchange of coenzyme preparations and mixed enzyme experiments established many reactions in common for the metabolism of serine by Cl. HF preparations and the synthesis of methionine in E. coli. A number of interesting dithiol compounds and invaluable advice as to their mode of synthesis and reactions were obtained from Drs. L. Stocken and J. Barltrop of the Univ. of Oxford. In München, new techniques of enzyme purification were learned and valuable advice obtained from the organic chemists on amino acid and sulfur chemistry.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-35
SERIAL NO.

16. NONE
PUBLICATIONS

17. NONE
HONORS AND AWARDS

7. a Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute 2. Laboratory of Cellular Physiology and Metabolism
INSTITUTE LABORATORY

3. _____ 4. _____ 5. NHI-36
SECTION LOCATION (IF OTHER THAN BETHESDA) SERIAL NO.

6. Synthesis, Isolation and Characterization of Compounds of Biological Interest
PROJECT TITLE

7. H. Todd Miles
PRINCIPAL INVESTIGATOR

8. NONE
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Synthesis, Isolation and Characterization of Compounds of Biological Interest.

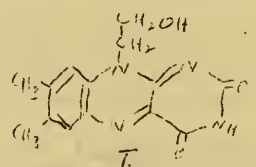
Objectives: The purpose of this research is to synthesize and to study the physical and chemical properties of compounds of special biochemical interest. In addition substances accumulating as biological intermediates are isolated and identified.

Methods Employed and Major Findings: An investigation of the tautomeric structure of pyrimidine nucleosides and nucleotides was made using the method of infrared spectroscopy in deuterium oxide solution. For this purpose a number of model compounds were prepared. The work showed that uridine and thymidine (and their 5'-phosphates) have the diketo structure in solution and that cytidine probably has the amino structure. This information should be useful in developing or confirming ideas of structure of the nucleic acids and in formulating reaction mechanisms of individual nucleotides. Crick and Watson have proposed a double helical structure for DNA in which the two strands of the helix are held together by hydrogen bonding between the purine and pyrimidine bases. Studies such as this one provide experimental evidence of tautomeric structure in solution and so indicate which hydrogen bonds can be formed.

In view of the great importance of riboflavin and reduced riboflavin in biological processes it was considered valuable to determine the spectrum of dihydroriboflavin over a wide spectral range. A study was therefore made of the ultraviolet spectrum of reduced riboflavin under a variety of pH conditions over the range from 220 to 600 mu. The reduction was carried out by three different methods: a) catalytic hydrogenation, followed by filtration of catalyst in the absence of air; b) use of potassium borohydride; c) a photochemical reduction in the presence of versene discovered recently by Merkel and Nickerson. The spectra found by the three methods

were essentially the same and differed in some respects from the only one reported previously (by the latter authors), and was over a wider range of wave lengths. The absorption maxima and extinction coefficients of riboflavin itself were also determined again on carefully purified samples.

Further confirmatory work in collaboration with E. R. Stadtman was done on the anaerobic bacterial breakdown products of riboflavin. The structure of the principal product was proved to be 6,7-dimethyl-9-(2'-hydroxyethyl)-iso-alloxazine, I. It was observed that red and green precipitates separated from the solution during the early course of the fermentation. These were shown, with a high degree of probability, to be free radical, quinhydrone-like complexes of the principal metabolic product, I. The green intermediate was isolated in the solid state. Although free radical intermediates in the biological reduction of riboflavin have often been postulated, this believed to be the first instance of the actual isolation of such a product from a biological source.



I.

Some preliminary experiments employing the hydroxamic analysis for thiol esters indicate that thiol esters are formed to some extent by the reaction of carboxylic acids and mercaptans in the presence of cyclohexylcarbodiimide. The method is not yet on a satisfactory basis, and further study will be needed to show whether it is a generally useful synthetic method A number of compounds of biological interest have been prepared for use in biochemical investigations.

Significance to HEART Research: This project is designed to provide fundamental information on the chemistry of biologically important compounds and to facilitate the studies on basic problems of intermediary metabolism carried out in the Laboratory of Cellular Physiology. It is expected that such information will contribute to the foundation upon which future medical advances in prevention and treatment of heart disease will be based.

Proposed Course of Project: Similar studies will be continued.

7. c

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-36
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-36
SERIAL NO.

16. Isolation and Characterization of 6,7-Dimethyl-9-(2'-hydroxy-ethyl)-isoalloxazine as a Bacterial Fermentation Product of Riboflavin, (with E. R. Stadtman), J. Am. Chem. Soc. 77, 5746 (1955).

17. NONE
HONORS AND AWARDS

8. a Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Laboratory of Cellular Physiology and Metabolism — LABORATORY OR BRANCH
3. _____ — SECTION
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-37 — SERIAL NO.

6. The Role of Folic Acid Derivatives in the Enzymic Conversion of Serine to Glycine. — PROJECT TITLE

7. Barbara W. Kalckar — PRINCIPAL INVESTIGATOR

8. NONE — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: The Role of Folic Acid Derivatives in the Enzymic Conversion of Serine to Glycine.

Objectives: To investigate the role of pteridine coenzymes in serine metabolism.

Methods Employed: Cell-free extracts capable of catalyzing the conversion of serine to glycine and a one-carbon compound are prepared from an anaerobic bacterium Clostridium HF. These enzyme preparations are used in studying the mechanism by which serine is cleaved to a one-carbon and a two-carbon compound (glycine). Methods used in analyzing complex intermediates involved in this reaction include: (1) The use of radioactive compounds, in particular C^{14}-serine and P^{32} orthophosphate (2) The quantitative determination of amino acids by conversion to their respective DNP-derivatives and subsequent chromatography (3) The quantitative determination of pentose, and of stable and labile phosphate.

Major Findings: Pteridine cofactors are required for the conversion of serine to glycine. A number of folic acid derivatives have been isolated and partially identified from boiled extracts of Clostridium cylindrosporum. Three new facts of interest in the field of a one-carbon metabolism appeared from a chemical examination of these "Co C" coenzymes: (1) They are polyglutamates, containing 3 or 6 glutamic acid residues; (2) They contain varying amounts of certain amino acids involved in serine metabolism; (3) Some contain pentose and phosphate.

Attempts were then made to synthesize these complex folic acid derivatives enzymatically from teropterin (diglutamyl folic acid). It was found that such compounds are formed, and that their formation is dependent upon teropterin, PO_4, serine and enzyme, but not upon Mn^{++}, B_6 and DPN, which are required in addition for glycine formation. These compounds are catalytically

active as coenzymes in the formation of glycine from serine, and preliminary experiments indicate that (1) they liberate glycine on acid hydrolysis and that (2) they contain phosphate, from studies on P_{32} incorporation.

Significance to HEART Research: There is ample evidence that folic acid derivatives play an important part in intermediary metabolism. One of their major roles is that of coenzymes in the many reactions involving one-carbon compounds. The present research project, as a part of the fundamental research program of the Heart Institute, is directed towards the accumulation of basic information in this general area of biochemistry. Since it is known that the conversion of serine to glycine is associated with the formation of a one-carbon compound folic acid complex, a detailed study of this reaction will help to elucidate the mechanism by which folic acid derivatives are used in biosynthetic reations. Such information should lead ultimately to a better understanding of heart metabolism and of intermediary metabolism in general.

Proposed Course of Project: These studies will be continued, with particular emphasis on the enzymic biosynthesis of P^{32} labeled pteridine coenzymes, with subsequent analyses of amino acid, phosphate and pentose content.

8. c Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-37
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Analysis of NIH Program Acitivities

Honors, Awards, and Publications Sheet

15. NHI-37
SERIAL NO.

16. A New Cofactor in the Conversion of Serine to Glycine, Biochim. et Biophys. Acta 16, 165 (1955).

Polyglutamyl Pteridine Coenzymes, J. Am. Chem. Soc. 77, 3930 (1955).

In Press:

The Role of Polyglutamyl Pteridine Coenzymes in Serine Metabolism
I. Cofactor Requirements in the Conversion of Serine to Glycine
II. A Comparison of Various Pteridine Derivatives
(To appear in J. Biol. Chem. April, 1956).

17. NONE
HONORS AND AWARDS

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Cellular Physiology and Metabolism
LABORATORY OR BRANCH

3. Metabolism
SECTION

4.

5. NHI 38 C
SERIAL NO.

6. Serum lipoproteins
PROJECT TITLE

7. Joseph H. Bragdon, Richard J. Havel
PRINCIPAL INVESTIGATORS

8. Dorothea Chu, Carlos Schultz
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: The study of serum lipids and lipoproteins in health and disease

Objectives: To study serum lipoproteins, their character, metabolism, and relation to atherosclerosis.

Methods Employed: Various lipoprotein classes are separated from serum by differential ultracentrifugation. The chemical composition of the classes is then determined. In some experiments isotopically labeled lipoproteins are used.

Patient Material: (1955 calendar year)

		No.	Average Stay Days
Admissions:	Adult males	4	45
	Adult females	1	45
Outpatient	Number of patients	6	
	Number of visits	24 (4 each)	

Major Findings: In humans a high-fat meal is followed by increases not only in the low-density lipoproteins, but also in the high-density classes, particularly in the phospholipid content. Fasting produces little or no change during 24 hours. Carbohydrate feeding results in striking reductions in low-density lipoprotein levels. A significant reduction in serum lipids has also been observed in rats fed carbohydrate. Carbohydrate-fed rats do not respond to protamine with the hyperlipemia observed in fasting rats.

Three siblings with idiopathic hyperlipemia have been shown to produce very little lipoprotein lipase following heparin injection. Other patients with apparently the same syndrome have responded in a normal manner.

Major Findings, Continued:

Short-term testosterone administration to children resulted in a rapid fall in high-density lipoproteins.

Following the oral administration of labeled cholesterol, the serum lipoproteins were studied. Significant differences were observed in the specific activity of different lipoprotein classes. Free cholesterol exchanged rapidly among the classes, whereas ester cholesterol did not.

Dog chyle chylomicrons, labeled with cholesterol, were injected into dogs. Activity disappeared rapidly from the blood stream. Appearance of activity in other lipoprotein classes was delayed.

Rat chyle chylomicrons, labeled with palmitic acid, were injected into rats. Activity disappeared rapidly from the blood stream, and was found predominantly in liver and spleen. The rate of clearance appears to be unrelated to the caloric balance of the rat.

Significance to HEART Research: Evidence continues to accumulate that serum lipoproteins play a role in the pathogenesis of atherosclerosis. It therefore becomes important to study lipoprotein metabolism.

Proposed Course of Project: To continue along same lines.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-38 C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-38 C
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Havel, R.J., Eder, H.A., and Bragdon, J.H., The Distribution and Chemical Composition of Ultracentrifugally Separated Lipoproteins in Human Serum. J. Clin. Invest. 34, 1345, 1955.

Bragdon, J.H., and Mickelsen, O., Experimental Atherosclerosis in the Rat. Am. J. Path. 31, 965, 1955.

17. HONORS AND AWARDS

None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Cellular Physiology and Metabolism — LABORATORY OR BRANCH
3. Metabolism — SECTION
4.
5. NHI-39 C — SERIAL NO.
6. Studies on Clinical Nephritis — PROJECT TITLE
7. J. H. Baxter and Howard C. Goodman — PRINCIPAL INVESTIGATORS
8. Joan Swantko — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION

Objectives: To study patients with nephritis and nephrotic syndrome in regard to (a) pathogenesis of disease, (b) metabolic abnormalities, and (c) best methods and results of therapy, particularly with steroid hormones.

Methods Employed: Clinical and laboratory studies on patients.

Patient Material: (1955 calendar year)

		No.	Average Stay Days
Admissions:	Adult males	7	90
	Adult females	3	90
	Children male	7	90
	Children female	2	180
Outpatient	Number of patients	35-40	
	Number of visits	200-250	

Major Findings: We have now treated a total of 18 patients with nephrosis with hydrocortisone or prednisone, with the following results:

	Adults	Children
Complete remissions	2	6
Remission except for residual proteinuria	2	1
Remission " " " " and mild hypoalbuminemia	3	-
Failures (no change in proteinuria)	2 *	2
TOTALS	9	9

*Had diuresis on stopping therapy.

Major Findings, Continued:

The results indicate that satisfactory responses are not precluded by adult age, hematuria up to 100 million RBC per 24 hours, or moderate degrees of renal insufficiency.

It is our tentative conclusion that the proper dose of prednisone for therapy in nephrosis is approximately 40 mg. per day (presumably equivalent to about 160 mg. of hydrocortisone) for about a month, or longer in some cases. The drug is then gradually discontinued. Long term maintenance therapy is employed only in cases that relapse.

Significance to HEART Research: Nephritis and nephrosis often cause hypertension and cardiac disease. The present studies are directed toward a better understanding of these diseases and better methods of therapy.

Proposed Course of Project: The studies will be continued together with additional physiological and immunological studies.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-39 C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Drs. Orloff and Keitel of the Laboratory of Kidney & Electrolyte Metabolism have participated in studies of electrolytes and renal function in these patients. Dr. Liddle of the Branch of General Medicine has participated in studies on aldosterone excretion.

14. None

Honors, Awards, and Publications Sheet

15. NHI-39 C
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Baxter, J.H., Goodman, H.C., Keitel, H., and Orloff, J. Studies of Prednisone in the Nephrotic Syndrome. Proc. First Internatl. Conf. on Clin. and Metab. Effects of Meticorten and Meticortelone, NYC, May 31, 1955.

Keitel, H., Goodman, H.C., Gordon, R.S., Jr., Havel, R.J., and Baxter, J.H. The Nephrotic Syndrome in Congenital Quartan Malaria. Accepted for publication in J.A.M.A.

(Publication pertaining to study of tissue lipid changes in choline deficiency, described in previous reports:)

Baxter, J.H. and Goodman, H.C. Renal and Hepatic Lipid Alterations in Choline Deficiency: Relationship to Renal Necrosis. Proc. Soc. Exp. Biol. & Med. 1955, 89, 682.

17. HONORS AND AWARDS:

None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Cellular Physiology and Metabolism — LABORATORY OR BRANCH
3. Metabolism — SECTION
4.
5. NHI-40 — SERIAL NO.

6. Studies on Experimental Nephritis: The Nephrotoxic Antigen. — PROJECT TITLE

7. James H. Baxter and Howard C. Goodman — PRINCIPAL INVESTIGATORS

8. Joan Swantko — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To study (a) distribution of the nephrotoxic-serum antigen in renal and non-renal tissues of the rat, and (b) organ and species specificity of the antigen.

Methods Employed: Various rat tissues have been examined for nephrotoxic antigen by (a) testing ability of tissues to stimulate formation of nephrotoxic serum in rabbits, and (b) testing ability of tissues to absorb nephrotoxic antibodies from a standard preparation of anti-rat kidney serum.

Major Findings: Nephrotoxic antigen has been found not only in glomeruli but in various portions of kidney including medulla. Furthermore, it is present in large amounts in lung and placenta, and in smaller amounts in heart, intestine and many other organs. The antigen therefore is not organ specific. On the other hand, the nephrotoxic antigen in the rat differs serologically from that of other species. We believe that nephrotoxic serum produces damage principally in the kidney because the antigen in the kidney is present in large amounts in the glomerular basement membranes which are directly exposed to circulating antibodies.

Significance to HEART Research: There is a good deal of evidence to suggest that human nephritis may result from formation of auto-antibodies which attack the kidney. Although the experimental disease involves the production of antibodies in a second species, the disease resembles clinical nephritis in many respects.

Proposed Course of Project: Organs other than kidney will be studied more carefully for damage produced by anti-organ sera.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-40
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-40
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Baxter, J.H. Nephrotoxic-serum Antigen. Proc. 7th Ann. Conf. on the Nephrotic Syndrome, Boston, Oct. 21, 1955.

17. HONORS AND AWARDS:

None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Cellular Physiology and Metabolism
LABORATORY OR BRANCH

3. Metabolism
SECTION

4.

5. NHI-41
SERIAL NO.

6. Studies on Experimental Nephritis: Soluble Protective Factor
PROJECT TITLE

7. Howard C. Goodman and James H. Baxter
PRINCIPAL INVESTIGATORS

8. Joan Swantko
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Preparation and characterization of a soluble factor produced by trypsin digestion of nephrotoxic antigen which is capable of neutralizing nephrotoxic serum in vitro and in vivo.

Methods Employed: Organ homogenates are digested at 37° for 3 hours with trypsin and then centrifuged in the Spinco ultracentrifuge at 66,000 g. The supernatant solution is then tested for presence of antigen or antigen derivative by our standard rat assay method.

Major Findings: Soluble protective factor has been prepared by trypsin digestion of homologous kidney, lung, skeletal muscle and heart. Characteristics of the material were described in previous report. Our purest preparation so far contains 0.1 mg. protein per protective dose.

Significance to HEART Research: It is conceivable that a factor might be prepared from human kidney by similar methods which would be useful in treatment of nephritis.

Proposed Course of Project: Further work on purification and characterization are contemplated.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-41
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-41
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Goodman, H.C. Soluble Protective Substance. Proc. 7th Ann. Conf. on the Nephrotic Syndrome, Boston, Oct. 21, 1955.

17. HONORS AND AWARDS:

None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Cellular Physiology and Metabolism
LABORATORY OR BRANCH

3. Metabolism
SECTION

4.

5. NHI-42
SERIAL NO.

6. Studies on Renal Homotransplantation
PROJECT TITLE

7. Howard C. Goodman and James H. Baxter
PRINCIPAL INVESTIGATORS

8. Joan Swantko
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Studies on renal homotransplantation have been carried out to learn something of the nature of the immune response that results in rejection of the transplanted kidney, and to study the effects of infusions of extracts of trypsin-digested kidney on survival of the transplanted kidney.

Methods Employed: Transplantation of the donor kidney to the neck of the recipient dog is performed by anastomosing the renal artery with the carotid artery; the renal vein is anastomosed to the external jugular vein, and the ureter brought out through the skin.

Major Findings: In 16 dogs studied so far, results indicate that intramuscular implantation of kidney tissue may serve to sensitize a recipient dog to donor kidney tissue. The effect of administration of extracts of trypsin-digested kidney on the survival of donor kidneys in sensitized dogs is being studied.

Significance to HEART Research: At present it is impossible to transplant human kidneys successfully except in the case of identical twins. Transplantation of kidneys is the only hope in many patients with chronic kidney disease. It is hoped that these studies will help elucidate the mechanisms involved in rejection of transplanted kidneys.

Proposed Course of Project: Continuation of studies described.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-42
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Drs. Kay and Gaertner of the Surgery Branch have participated in this project.

14. None

Honors, Awards, and Publications Sheet

15. NHI-42
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Cellular Physiology and Metabolism — LABORATORY OR BRANCH
3. Metabolism — SECTION
4.
5. NHI-43 C — SERIAL NO.

6. (1) Enzymatic Degradation of Cholesterol. (2) Metabolism of (Radioactive) Lipids and Lipoproteins. (3) Studies on Cholesterol Inhibitors. — PROJECT TITLE

7. Donald S. Fredrickson (also clinical resident during this period) — PRINCIPAL INVESTIGATOR

8. Mr. Katsuto Ono — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: (1) Investigation of the enzymatic metabolism of cholesterol.
(2) A study of the metabolism of lipoproteins labeled with radioactive lipid.
(3) In vitro and clinical trial of compounds designed to lower plasma cholesterol levels.

Methods Employed: Radioactive cholesterol is incubated with cell-free preparations from liver and the products of catabolism are recovered and characterized. Lipoproteins tagged with radioactive lipid are isolated from donor animals or humans and their fate in recipients and behavior with respect to other lipoprotein classes is studied. Compounds are tested in vitro for effect on cholesterol synthesis. After suitable toxicity studies, clinical trial in patients is made.

Patient Material: (6 mos. of 1955 calendar year)

		No.	Average Stay Days
Admissions:	Adult males	4	92
	Adult females	3	53
Outpatient:	Number of patients	25	
	Number of visits	125	

Major Findings: (1) Mouse liver mitochondria have been found capable of the production of at least four different acidic steroids from cholesterol-C^{14}. Other than small amounts of lithocholic acid, these acids are similar, but not identical to the bile acids common to mammalian bile. In addition to esters, another enzymatically produced derivative, possibly an intermediate, has been identified as 25-Hydroxycholesterol.

Major Findings, Continued:

(2) The neutral fat moiety of chylomicrons has been found to be rapidly hydrolyzed during the phase of "clearing". The turnover of plasma unesterified fatty acids in the dog has been found compatible with the involvement of the enzyme, 'lipoprotein lipase' during clearing. Marked differences in exchangeability of free and ester cholesterol between lipoproteins have led to a clearer understanding of the lipoprotein structure. Differential rates of "clearing" of chylomicron lipid moieties have been discovered.

(3) Extensive studies with aromatic acid derivatives, used widely in Europe, have indicated the mechanism and potency of their in vitro activity, but have shown them to be of little clinical value.

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.

Proposed Course of Project: Basically, these same studies will be continued and extended. Emphasis is being shifted to development of techniques for identification of fatty acids on a micro-analytical scale, to facilitate study of the metabolism of these moieties.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-43 C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-43 C
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Steinberg, D. and Fredrickson, D.S., Inhibition of Lipid Synthesis by Alpha-N-Butyric Acid and Related Compounds. Proc. Soc. Exper. Med. & Biol. 90, 232 (1955).

OTHER PUBLICATIONS NOT RELATED TO THIS PROJECT:

Fredrickson, D.S., Ganong, W.F., and Hume, D.W., Thyroid Uptake of Radioactive Iodine in the Dog, Effect of Diet Hypophysectomy and TSH. Proc. Soc. Exper. Med. & Biol. 89, 416 (1955).

Ganong, W.F., Fredrickson, D.S. and Hume, D.W., The Effect of Hypothalmic Lesions on Thyroid Function in the Dog. Endocrinology 57, 355 (1955).

17. HONORS AND AWARDS:

None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Cellular Physiology and Metabolism — LABORATORY OR BRANCH
3. Metabolism — SECTION
4.
5. NHI-44 — SERIAL NO.
6. Structural muscle proteins and their interactions. — PROJECT TITLE
7. Elemer Mihalyi — PRINCIPAL INVESTIGATOR
8. Irene M. Knoller, Edwin F. Wilson — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION

Objectives: To study the various structural muscle proteins from the physico-chemical and chemical points of view, to investigate their interrelation in muscle extracts and their interaction in the isolated state.

Methods Employed: Chemical, ultracentrifugal and spectroscopic analysis of muscle extracts obtained by different procedures. Dissociation of the myosin molecule by proteolytic enzymes, chemical, or physical agents into its components and characterization of the latter by physico-chemical and chemical methods.

Major Findings: Myosin preparations contain a ribonucleic acid impurity, which is responsible for the phosphorus content of the protein. This nucleic acid has been isolated and characterized. A heat stable protein was also separated from myosin, which has no aromatic amino acids. The viscosity response of Myosin B (actomyosin) preparations to adenosinetriphosphate could not be correlated to their optical properties, a result which is in contradiction with the actomyosin theory. The ultraviolet absorption spectrum of myosin could be explained on the basis of the amino acid composition and nucleic acid content of the protein.

Significance to HEART Research: In the present investigations rabbit skeletal muscle was used. The findings with this material may help to understand function and disease of the heart muscle. On the other hand, experience gained with skeletal muscle will be of help in the future study of the apparently more complicated heart muscle.

Proposed Course of Project: Characterization of the heat stable protein will be continued. The main objective of the work will be, however, the transformation of myosin A to myosin B. It seems possible that divalent cations play a part in this transformation and the nature of the proteins involved, beside myosin itself, remains to be elucidated.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-44
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

The sedimentation studies on the ribonucleic acid were done in collaboration with Dr. Dan F. Bradley in the National Institute of Mental Health.

14. None.

Honors, Awards, and Publications Sheet

15. NHI-44
SERIAL NO.

16. None.

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Cellular Physiology and Metabolism
LABORATORY OR BRANCH

3. Metabolism
SECTION

4.

5. NHI-45
SERIAL NO.

6. The Inactivation of Insulin by an Enzyme from Rat Liver
PROJECT TITLE

7. Martha Vaughan
PRINCIPAL INVESTIGATOR

8. Jane Logan
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To further purify the enzyme. To determine the nature of the end products of insulin degradation in the system in order to establish the bond specificity of the enzyme. To study various inhibitors of the enzyme which, if also active in vivo, might be of physiologic or pharmacologic interest in addition to their usefulness as tools in the study of the mechanism of enzyme action.

Methods Employed: Fractionation of proteins from liver extracts. Assay of "insulinase" activity or of inhibitor activity by measuring formation of trichloroacetic acid soluble I^{131} from I^{131} labeled insulin, liberation of non-protein nitrogen from or inactivation of insulin. Identification of peptides formed from insulin by chromatography and other techniques.

Major Findings: Some further purification of the enzyme has been achieved. The degradation of insulin evidently involves cleavage at many points in the molecule. The time sequence of appearance of new N-terminal amino acids during digestion of the isolated B chain of oxidized insulin has been studied. Several dithiohydantoin compounds have been found to be potent inhibitors of the enzyme and a series of other types of sulfur and non-sulfur containing compounds have been tested and found to be lacking in inhibitory activity.

Significance to HEART Research: This project is a part of the basic research program of the National Heart Institute. Although it does not relate directly to heart disease the knowledge obtained concerning cellular metabolism should contribute to our understanding of cardiovascular function.

Proposed Course of Project: In addition to the continuation of studies on the sites of enzymatic cleavage in the insulin molecule it is planned to test for enzyme inhibitory activity a large number of compounds of many different types with the hope of obtaining one or more inhibitors which may be used to inhibit the enzyme in vivo. Drugs of this type should provide a useful tool for further studies directed toward an evaluation of the postulated role of this enzyme in insulin metabolism under physiological and pathological conditions.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-45
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT:

None.

14. None

Honors, Awards, and Publications Sheet

15. NHI-45
SERIAL NO.

16. None.

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Cellular Physiology and Metabolism
LABORATORY OR BRANCH

3. Metabolism
SECTION

4.

5. NHI-45(1)
SERIAL NO.

6. Assay of serum transaminase as a diagnostic aid in coronary artery disease.
PROJECT TITLE

7. Daniel Steinberg
PRINCIPAL INVESTIGATOR

8. Don Baldwin
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To perfect the enzymatic assay and to evaluate it as a diagnostic tool.

Methods Employed: Glutamic-oxalacetic transaminase is assayed by a modification of the method of LaDue. The reaction is coupled with malic dehydrogenase and the rate of disappearance of DPNH is determined. Patient material for this study is supplied by the Cardiac Service at George Washington University Hospital.

Patient Material: No patients have been admitted to the Clinical Center specifically for this project. About 25 Clinical Center patients, under observation for other purposes, have been followed.

Major Findings: A simplified, clinically applicable assay procedure has been perfected.

In a series of over 200 cases the value of serum transaminase as a diagnostic tool has been firmly established. It has been of particular value in giving objective evidence of infarction when the electrocardiographic picture has been confused because of bundle-branch block, previous myocardial infarction, pericarditis, transient myocardial ischemia, and digitalis effects. In addition, it is suggested that a number of cases showing only ST-T changes do in fact suffer myocardial necrosis difficult to affirm on the basis of EKG changes only. In 15 cases coming to autopsy, serum transaminase has proved in over 90% of the cases to be a valid and reliable index of myocardial necrosis.

Significance to HEART Research: The importance of establishing or ruling out the diagnosis of myocardial infarction is clear. Even with the electrocardiogram there is not infrequently an unresolvable doubt as to whether infarction has occurred or whether the patient has pain due to coronary insufficiency without infarction. The modified assay procedure makes this diagnostic tool available to the practicing cardiologist.

Proposed Course of Project: Completed.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-45(1)
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

George Washington University Hospital - Dr. Bernard Ostrow, Dr. John M. Evans and Dr. Howard Ticktin.

14. None.

Honors, Awards, and Publication Sheet

15. NHI-45(1)
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Steinberg, D., and Ostrow, B., Serum Transaminase as a Measure of Myocardial Necrosis. Proc. Soc. Exper. Biol. and Med., 89, 31-34, 1955.

17. HONORS AND AWARDS:

None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Cellular Physiology and Metabolism
LABORATORY OR BRANCH

3. Metabolism
SECTION

4.

5. NHI-46
SERIAL NO.

6. Studies on the Mechanism of Protein Synthesis and Degradation.
PROJECT TITLE

7. Daniel Steinberg and Martha Vaughan
PRINCIPAL INVESTIGATORS

8. Fred Czech, Jane Logan, Don Baldwin and James Brophy
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To elucidate the mechanism by which amino acids are assembled in the proper sequence to form specific proteins. To determine the nature of the "proteolytic" processes which are implied as a constant concomitant of synthesis in the dynamic state.

Methods Employed: C^{14}-labeled amino acids are incubated *in vitro* with tissue slices or homogenates under various conditions and the pattern of uptake into specific proteins or the total tissue protein is determined. Protein degradation *in vitro* under physiologic conditions is followed either by measurements of non-protein nitrogen or by measurement of the release of C^{14} amino acids from tissues previously tagged by injection of labeled amino acids into the whole animal.

Major Findings: The studies previously reported have been extended showing that the inhibition of protein breakdown by o- and p-fluorophenylalanine is general. Similar findings are obtained in liver slices and in kidney slices. Catheptic activity in liver homogenates is influenced slightly or not at all by the concentration of inhibitors effecting a 50% inhibition of protein breakdown in slices. These findings are presumptive evidence for a mechanism of protein breakdown other than catheptic hydrolysis.

Significance to HEART Research: This project is part of the basic research program of the National Heart Institute. While it does not relate specifically to heart disease the knowledge gained relative to cellular metabolism in general will contribute to furthering our understanding specifically of cardiovascular function.

Proposed Course of Project: Efforts to find a suitable cell-free system have not been successful using the endogenous tissue proteins as substrate. Attempts to use added iodinated protein as substrate are planned.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-46 _____
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None.

14. None

Honors, Awards, and Publications Sheet

15. NHI-46
SERIAL NO.

16. None.

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Cellular Physiology and Metabolism
LABORATORY OR BRANCH

3. Metabolism
SECTION

4.

5. NHI-47
SERIAL NO.

6. Studies on the Chemistry and Metabolism of Serum Lipoproteins.
PROJECT TITLE

7. Daniel Steinberg, Joel Avigan, Howard Eder and Robert Redfield
PRINCIPAL INVESTIGATORS

8. Edwin Wilson, Juanita Page, Mary Ann Hurley
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To further characterize the classes of serum lipoproteins and to clarify their metabolic relationships and functions.

Methods Employed: (a) Preparation of intrinsically labeled lipoproteins of high specific radioactivity by the feeding of uniformly labeled algal protein. The latter was prepared by growing Chlorella in $C^{14}O_2$. Purification of the specific lipoprotein fractions by ultracentrifugal methods. Injection of these biologically labeled materials into recipient animals for determination of half-time and metabolic fate.

(b) End group analysis using DNFB technique of Sanger for N-terminal groups and carboxypeptidase digestion for C-terminal groups.

Major Findings: (a) Interconversion of beta$_1$-lipoprotein and alpha$_1$-lipoprotein occurs slowly and to a small extent if it occurs at all. Similarly, proteins in the nonlipoprotein fractions of serum are not converted to lipoproteins at any considerable rate. The half-lives of serum lipoproteins while somewhat shorter than that of other serum proteins does not differ strikingly from them (3 to 5 days). If it is assumed that the triglyceride moiety of the beta$_1$-lipoprotein and of the alpha$_1$-lipoprotein has a turnover rate not radically greater than that of the protein moiety it must be concluded that these molecules play a quantitatively unimportant role in neutral fat transport.

(b) The N-terminal residue in beta$_1$-lipoprotein has been shown to be glutamic acid while that in the alpha$_1$-lipoprotein is aspartic acid. This clear-cut chemical difference in the protein molecules would in itself suggest their metabolic independence in agreement with the above isotope results. The beta$_1$-lipoprotein does not appear to be attacked by carboxypeptidase even after partial removal of the lipid. The C-terminal residue in the alpha$_1$-lipoprotein is probably leucine but final identification has not been completed.

Significance to HEART research: Because of the implication of lipoproteins in the genesis of atherosclerosis, an understanding of the fundamental chemistry and physiology of these molecules is clearly a first consideration.

Proposed Course of Project: The nature of the attachment of the lipid moiety to serum lipoproteins will be investigated. Further physical chemical studies of the spectrum of lipoproteins found in the circulation will be carried out.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-47
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publication Sheet

15. NHI-47
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Cellular Physiology and Metabolism
LABORATORY OR BRANCH

3. Metabolism
SECTION

4.

5. NHI-48C
SERIAL NO.

6. Studies on Inhibitors of Cholesterol Synthesis
PROJECT TITLE

7. Daniel Steinberg and Donald S. Fredrickson
PRINCIPAL INVESTIGATORS

8. Fred Czech and Katsuto Ono
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To investigate inhibitors of cholesterol synthesis in terms of mechanisms of action and possible clinical applications.

Methods Employed: In vitro studies to determine effects on the major metabolic pathways in addition to cholesterol synthesis. Animal studies to determine any possible toxic effects and to determine the extent of lowering of blood cholesterol that can be achieved. Clinical evaluation of compounds demonstrated to be effective in vitro and nontoxic in animal studies.

Patient Material: (6 mos. of 1955 calendar year)

		No.	Average Stay Days
Admissions:	Adult males	4	92
	Adult females	3	53
Outpatient:	Number of patients	25	
	Number of visits	125	

Major Findings: The mechanism of action of alpha-phenylbutyric acid and beta-phenylpropionic acid has been established as an inhibition of acetate activation, acetoacetate formation or both. Contrary to expectations and in contradiction of published results in the French literature, beta-phenylpropionic acid has been without effect on the blood cholesterol level in a series of 20 cases.

Delta4-cholestenone fed to rats at a level of 1% in the diet markedly depresses serum cholesterol levels. In addition, however, it causes striking adrenal hypertrophy, the cause of which is currently under investigation.

Significance to HEART RESEARCH: Since over 50% of the daily cholesterol turnover arises by endogenous synthesis a well-tolerated effective inhibitor may depress serum cholesterol levels and in this way slow the development of atherosclerosis.

Proposed Course of Research: Analogues of compounds in the cholesterol biosynthetic pathway will be examined in the manner described above. Clinical studies will be carried out on promising compounds.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-48C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None.

14. None.

Honors, Awards, and Publications Sheet

15. NHI-48C
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Steinberg, D., and Fredrickson, D.S. Inhibition of Lipid Synthesis by Alpha-phenyl-n-butyrate and Related Compounds. Proc. Soc. Exper. Biol. and Med., 90: 232, 1955.

17. HONORS AND AWARDS

None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Cellular Physiology and Metabolism — LABORATORY OR BRANCH
3. Metabolism — SECTION
4.
5. NHI-49 — SERIAL NO.
6. Mechanisms of enzymatic decarboxylation. — PROJECT TITLE
7. Daniel Steinberg and Simon Rothberg — PRINCIPAL INVESTIGATORS
8. — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION

Objectives: To determine the molecular mechanisms of enzymatic decarboxylation by studying the role of water in the formation of the enzyme substrate complex and in the labilization of the carbon-to-carbon bond.

Methods Employed: Determination by mass spectrometry of the O^{18} incorporation into substrate and products during enzymatic decarboxylation.

Major Findings: Enzymatic decarboxylation of aspartic acid, glutamic acid and tyrosine occurs without the participation of H_2O. The formation of a hydrated intermediate prior to removal of CO_2, proposed by some as the mechanism for decarboxylation, appears to be ruled out. Likewise, formation of an acyl-enzyme complex is ruled out. Decarboxylation of malonic acid, a coenzyme A dependent decarboxylation, occurs beta to the coenzyme A linkage and does not lead to O^{18} incorporation from the medium. In cooperation with Dr. Hayaishi and Dr. Katagiri, it has been shown that molecular oxygen is involved in catechol oxidation.

Significance to HEART Research: This project is a part of the basic research program of the NHI. While it does not relate specifically to heart disease the knowledge gained relative to cellular metabolism in general will contribute to furthering our understanding specifically of cardiovascular function.

Proposed Course of Project: This project is approaching completion and should not run more than another 2 or 3 months. During this time the effects of inhibitors and of pyridoxal phosphate will be studied.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-49
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Dr. Osamu Hayaishi and Dr. Masayuki Katagiri of NIAMD.

14. None.

Honors, Awards, and Publications Sheet

15. NHI-49
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Hayaishi, O., Katagiri, M., and Rothberg, S. Mechanism of Pyrocatechase action. J.A.C.S. 77, 6250 (1955).

17. HONORS AND AWARDS:

None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Cellular Physiology and Metabolism — LABORATORY OR BRANCH
3. Metabolism
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-500 — SERIAL NO.
6. Metabolism of Plasma Unesterified Fatty Acids — PROJECT TITLE
7. Robert S. Gordon, Jr., M.D. — PRINCIPAL INVESTIGATOR(S)
8. Amelia Cherkes — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To evaluate the metabolic significance of the small amount of unesterified fatty acid present in all plasmas.

Methods employed: Chemical analysis of plasmas for their content of unesterified fatty acid. The latter is studied in terms of its response to various metabolic stimuli.

Patient Material: (1955 calendar year)

		No.	Average Stay Days
Admissions:	Adult males	3	50
Outpatient	Number of patients	3	
	Number of visits	11	

Major Findings: Findings with respect to fatty acid concentrations now seem to indicate that plasma unesterified fatty acid is the major pathway for the transport of fat from storage depots to metabolically active tissues during fasting. The mechanism is under hormonal control, with obvious effects due to insulin and epinephrine. Its relation to the transport of dietary fat remains to be elucidated.

Significance to HEART Research: It is hoped that study of the mechanisms of fat transport will eventually lead to an understanding of the etiology of atherosclerosis.

Proposed Course of Project: It is hoped that we will be able to elucidate the hormonal mechanisms controlling unesterified fatty acid release, and to discover the relationship between this fraction of plasma and

Analysis of NIH Program Activities

Project Description Sheet

NHI-50C
SERIAL NO.

PROJECT TITLE: Metabolism of Plasma Unesterified Fatty Acids

9. PROJECT DESCRIPTION: (continued)

the postcibal lipemia which is so familiar. It is also hoped that experiments with isotopically labeled fatty acids can be carried out in human subjects. A preliminary study was begun to confirm Ahren's finding that serum cholesterol is affected by the type of fat in the diet of a normal human subject. Because of differences in the affinity of albumin for saturated and unsaturated fatty acid ions, it is a possibility that the influence of diet on cholesterol levels is in some way related to effects on unesterified fatty acids. Further investigation of this possibility is planned if suitable subjects become available.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-50C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications

15. NHI-50C
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Gordon, R.S., Jr., Interaction between Oleate and the Lipoproteins of Human Serum. J. Clin. Invest. 34, No. 3, pp. 477-484, March 1955.

In Press:

Gordon, R.S., Jr., and Cherkes, Amelia. Unesterified fatty acids in human blood plasma. J. Clin. Invest.

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
a. Cellular Physiology and Metabolism — LABORATORY OR BRANCH

3. Metabolism — SECTION
4. — LOCATION(IF OTHER THAN BETHESDA)
5. NHI-51C — SERIAL NO.

6. Electrophoretic Studies of Serum Proteins
PROJECT TITLE

7. Robert S. Gordon, Jr., M. D.
PRINCIPAL INVESTIGATOR(S)

8. None.

9. PROJECT DESCRIPTION

Objectives: To utilize the great accuracy of the Aminco Model B Electrophoresis Apparatus in clinical research, both on our own studies and in collaboration with the other Institutes.

Methods employed: Operation of the electrophoresis apparatus.

Patient Material: (1955 Calendar Year)

		No.	Average Stay Days
Admissions:	Adult females	1	33
Outpatient:	Number of patients	1	
	Number of visits	1	

Major Findings: The normal values of serum protein concentrations for young human subjects have been established and are in publication. In addition, analyses have been made in detail in several conditions of abnormal serum protein metabolism -- agammaglobulinemia, and one case of essential hypoalbuminemia. From these data it has been possible to calculate turnover rates for the exogenously supplied replacement proteins.

Significance to HEART Research: The electrophoretic method is of general usefulness in clinical pathology. Having it established in the Heart Institute makes it possible to analyze sera for protein disturbances in any of the clinical services. The results obtained to date are of real academic interest. This program is in line with the NHI policy of promoting vigorous basic research.

Proposed Course of Project: The protein studies will continue as at present with special emphasis on albumin, which serves normally as the

Analysis of NIH Program Activities

Project Description Sheet

NHI-51C
SERIAL NO.

PROJECT TITLE: Electrophoretic Studies of Serum Proteins

9. PROJECT DESCRIPTION: (continued)

vehicle for unesterified fatty acid. In addition, the apparatus will be available for the convenience of others who wish to analyze sera or protein preparations of various types.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-51C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Microbiological Institutes (cooperating on study of agammaglobulinemia).

Clinical Pathology Department (cooperating on studies of albumin).

14. None

Honors, Awards, and Publications

15. NHI-51C
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

In Press:

Gordon, R.S., Jr. Observations on the Electrophoretic Analysis of Normal Human Serum. Clinical Chemistry.

Martin, C.M., Gordon, R.S., and McCullough, N.B. Acquired Hypogammaglobulinemia: Report of a Case with Clinical and Experimental Studies. N.E.J.M.

Keitel, H.G., Goodman, H., Havel, R.J., Gordon, R.S., and Baxter, J.H. The Nephrotic Syndrome in Congenital Quartan Malaria. J.A.M.A.

17. None.

ANALYSIS OF NIH PROGRAM ACTIVITIES

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Cardiovascular Hemodynamics (LABORATORY OR BRANCH)

3. None 4. None 5. NHI-52 (SERIAL NO.)

6. Mechanisms of the Circulatory Derangement During High Levels of Positive Pressure Breathing. (PROJECT TITLE)

7. J.T. Binion, M.D., W.L. Morgan, Jr., M.D. and S.J. Sarnoff, M.D. (PRINCIPAL INVESTIGATORS)

8. None

9. PROJECT DESCRIPTION

Objectives: To ascertain (a) the nature of the circulatory derangement as a result of which, under high levels of positive pressure breathing (50 to 70 cm H_2O), arterial pressure and cardiac output fall and venous pressure rises.

Major Findings: Seventeen dogs were studied to substantiate the previous finding that Aramine will restore the circulation during high levels of positive pressure breathing. Seven dogs, wrapped with an external counterpressure suit, were given Aramine in single doses of 0.2 mg/k intravenously after their blood pressures had fallen to low levels during an average airway pressure at 57-76 cm H_2O. Seven other dogs without external counterpressure were subjected to airway pressures from 35 to 48 cm H_2O, and were given Aramine in a like manner. Three dogs without suits were given Aramine intramuscularly in doses of 0.5 mg/k when they became severely hypotensive. All seventeen dogs responded with a rise in arterial pressure and were able to tolerate substantially longer periods at the given positive airway pressure.

Eighteen dogs were studied to elucidate the mechanism of the circulatory depression during positive pressure breathing. No satisfactory method was found to measure interpleural pressures with high levels of positive pressure breathing. Angiograms (cineradiofluorography) during positive pressure without a counterpressure suit demonstrated a smaller cardiac shadow, a distended vena cava below the diaphragm and decrease in caval size within the chest. However, measurements of pressures across the diaphragm showed no evidence of obstruction at that level. This supports our hypothesis that positive pressure breathing leads to displacement of the blood from the chest to periphery due to the high intra-thoracic pressure but with no isolated site of vascular obstruction in the chest.

It is concluded that sympathomimetic agents such as Aramine improve the depressed circulation during high levels of positive pressure breathing by (a) constricting peripheral vessels, including veins, and replacing displaced blood from the periphery back to lung and thereby providing a higher filling pressure for ventricular contraction; and (b) by elevating the ventricular function curve and thereby counteracting the cardiac tamponade effect of positive pressure breathing.

When a counterpressure suit was applied to the dog, there was a considerable elevation in intra-abdominal caval pressure that was obliterated by placing a rigid perforated tube in the abdomino-thoracic inferior vena cava. The circulatory depression of positive pressure breathing was not diminished by this means of preventing vena caval obstruction.

Proposed Course of Project: To correlate the observed hemodynamic phenomena quantitatively with changes in pulmonary blood volume as estimated by the blue dye injection technique.

Significance: These studies have contributed substantially to our understanding of the nature of circulatory derangement under high levels of positive pressure breathing, particularly as to the role played by cardiac tamponade; it is also shown that the use of sympathomimetic agents can effectively counteract the circulatory derangement for significant periods of time.

1.b

ANALYSIS OF NIH PROGRAM ACTIVITIES

Budget Data Sheet

10. NHI-52
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-52
SERIAL NO.

16. None

17. None

2.a ANALYSIS OF NIH PROGRAM ACTIVITIES

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Cardiovascular Hemodynamics (LABORATORY OR BRANCH)

3. None 4. None 5. NHI-53 (SERIAL NO.)

6. Studies in Cardiac Tamponade and the Effect Thereon of Sympathomimetic Agents. (PROJECT TITLE)

7. W.L. Morgan, Jr., M.D., J.T. Binion, M.D. and S.J. Sarnoff, M.D. (PRINCIPAL INVESTIGATORS)

8. None

9. PROJECT DESCRIPTION

Objectives: 1) To determine the effect of Aramine on Stroke work of the heart when stroke work is limited by limiting diastolic fiber length as in acute cardiac tamponade. 2) To evaluate the drug as a possible clinical therapeutic agent in emergency cardiac tamponade.

Major Findings: Six dogs have been used to determine simultaneous right and left ventricular function curves with at least four curves determined from each experiment: 1) One control ventricular function curve (stroke work vs. atrial pressure); 2) One curve following acute infusion of saline into the pericardial sac (60 to 120 cc) producing acute cardiac tamponade; 3) One curve following the intravenous injection of Aramine or Wyamine in the presence of the same degree of tamponade; and 4) a post control curve after release of tamponade. In each instance there was a higher curve following the sympathomimetic agent with the tamponade than with tamponade alone, i.e. more stroke work for a given atrial pressure on both the right and left sides of the heart.

Proposed Course of Project: Project completed.

Significance: These studies have demonstrated the physiologic fact that heart muscle can, when stimulated by sympathomimetic agents, be induced to contract more forcefully from the same restricted fiber length. They also suggest that the use of such agents as a holding maneuver in acute clinical cardiac tamponade would be of value.

2. b

ANALYSIS OF NIH PROGRAM ACTIVITIES

Budget Data Sheet

10. NHI-53
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-53
SERIAL NO.

16. None

17. None

ANALYSIS OF NIH PROGRAM ACTIVITIES

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Cardiovascular Hemodynamics (LABORATORY OR BRANCH)

3. None 4. None 5. NHI-54 (SERIAL NO.)

6. Heart Valve Replacement (PROJECT TITLE)

7. S.J. Sarnoff, M.D. and G.H. Welch, Jr., M.D. (PRINCIPAL INVESTIGATORS)

8. None

9. PROJECT DESCRIPTION

Objectives: To study ways of substituting prosthetic valves for diseased heart valves.

Major Findings: 1-a) The elastic silicone Silastic housing which has been substituted for the lucite housing in the hope of diminishing the red blood cell destruction produced by the latter has at least partially fulfilled this hope. The rate of red cell destruction with this valve is probably low enough to be acceptable for clinical use when used in the apical-aortic position. This was not true of the valve with the lucite housing. b) The valve does not give rise to clot. This suggests that this material will serve well as a vascular substitute as well as for valve housings. c) Some indication of the durability of this material is given by the fact that it has continued (and continues at this writing) to function well as a valve housing up to ten months. It is substantially quieter.

2) The nylon clip placed on the ascending aorta to roughly simulate aortic stenosis in earlier studies eroded through in some dogs. Through the use of pericardium to protect the aorta from the teeth of this clip, this difficulty has been eliminated and loss from this cause no longer occurs. This was previously an irritating source of trouble, especially since it would not be a part of the clinical procedure.

3) The major source of infection in our operation room has been ascertained and although infection has not been eliminated as a hazard, it has been greatly reduced.

4) Thrombus formation and embolism have been greatly reduced, although not eliminated, by wrapping the aortic tube-vessel junction with orlon mesh to occlude the cul-de-sac therein. This is more difficult in the dog than in man because of

structure size. That it can be made successful in man is indicated by the results of Hufnagel who has seen no emboli in man since adopting this procedure (up to 8 months).

5) We are, at long last, getting into a position to study the core of the problem. Three dogs have died of embolism in the last eleven dogs operated upon. The appearance of both the tube-vessel junctions and ventricular tube ends were such as to suggest that the emboli could have arisen from either site, and it is not possible to know which. Post-operative anticoagulant therapy may alleviate this source of difficulty. There is reason to hope, because of anatomic reasons, that this will be less of a hazard in man.

6) The procedure itself is now quite routine and is performed with ease.

7) Post-mortem observations (with Dr. Morrow) have been initiated with a view towards sizing prostheses for use in man. Similarly, the valve size for use in man is being given consideration.

8) Evidence for an increased rate of red cell destruction has been obtained in two of three patients in whom the Hufnagel valve was inserted for aortic insufficiency.

9) The Hufnagel valve (lucite ball in lucite housing) while acceptable for use in the Hufnagel position is clearly unsuitable for use in direct continuity with cardiac chambers. Such continuity is a prerequisite for definitive correction of valvular disease of the heart.

Proposed Course of Project: To investigate the feasibility of using end-to-side anastomosis between the valve by-pass and thoracic aorta and also a means of diminishing the embolic hazard.

Significance: It appears that the only residual road block to attempting to apply these techniques to man is the resolution of the embolic hazard. Should we be successful in doing so, a means of precise and definitive correction of valvular disease of the heart rather than the repair of diseased valves would be available

3. b

ANALYSIS OF NIH PROGRAM ACTIVITIES

Budget Data Sheet

10. NHI-54
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S).

Section on Hematology, Laboratory of Pathology and Histochemistry, National Institute of Arthritis and Metabolic Diseases.

14. None.

Honors, Awards, and Publications Sheet

15. NHI-54
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Sarnoff, S.J. and Case, R.B. Experimental by-pass of the aortic valve by valvular anastomosis between apex of the left ventricle and thoracic aorta. Henry Ford Hospital International Symposium on Cardiovascular Surgery; page 304-320, March, 1955.

Sarnoff, S.J. and Case, R.B. Physiologic considerations relating to the Hufnagel operation with special reference to postoperative anemia. Henry Ford Hospital International Symposium on Cardiovascular Surgery; page 328-340, March, 1955.

17. None.

ANALYSIS OF NIH PROGRAM ACTIVITIES

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Cardiovascular Hemodynamics (LABORATORY OR BRANCH)

3. None 4. None 5. NHI-55 (SERIAL NO.)

6. Neurogenic, Humoral and Metabolic Factors in Peripheral Vascular Hemodynamics
PROJECT TITLE

7. Robert P. Akers, Ph.D.
PRINCIPAL INVESTIGATOR

8. None

9. PROJECT DESCRIPTION

Project: The evaluation of the importance of certain humoral neurogenic and metabolic factors in the regulation of vasomotor activity of the terminal vascular bed.

Objectives: The objective of this project was to evaluate the usefulness of the hamster cheek pouch as a tool for bioassay of vasoactive substances.

Methods Employed: The threshold response of the hamster cheek pouch to a number of know vasoactive materials has been determined as they affect the vessels when applied topically. Attempts have been made to make the cheek pouch vessel responses specific of certain of these vasoactive drugs by using pharmacological blocking drugs.

Proposed Course of Project: Studies on the peripheral vascular system of the hamster have been terminated.

4.b

ANALYSIS OF NIH PROGRAM ACTIVITIES

Budget Data Sheet

10. NHI-55
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-55
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Robert P. Akers and B.W. Zweifach. The effect of vasoactive materials on the peripheral blood vessels of the hamster. Am. J. Physiol. In press.

17. None

5.a

ANALYSIS OF NIH PROGRAM ACTIVITIES

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Cardiovascular Hemodynamics — LABORATORY OR BRANCH
3. None
4. None
5. NHI-56 — SERIAL NO.

6. Studies on Tone of Isolated Blood Vessels — PROJECT TITLE

7. Edward Leonard, M.D. — PRINCIPAL INVESTIGATOR

8. None

9. PROJECT DESCRIPTION

Project: Studies on tone of isolated blood vessels.

Objectives: An attempt has been made to set up a model system to determine whether any known steroids affect arterial smooth muscle in a way that would increase the tone of the vessel. In the course of the investigation some observations on certain physiologic and pharmacologic properties of isolated blood vessels have also been made.

Methods: Experiments have been performed on helically cut vessel strips, suspended between two clamps, stimulated with alternating current pulses, and allowed to develop tension isometrically. The tension is exerted against the cantilever of a sensitive strain gage. Effects of various interventions on the contractile response to a standard electrical stimulus are studied.

Major Findings: The major findings have to do with the effects of potassium and certain steroids on the muscle tone. In K+ free Krebs solution the muscle slowly goes into contracture, a phenomenon which is hastened if the strip is stimulated. In normal Krebs solution a similar effect can be obtained with strophanthin, a cardiac glycoside. Desoxycorticosterone and progesterone do not cause contracture, and in fact can prevent the contracture which normally occurs in K+ free solutions.

Significance: Examples of steroid action on vascular smooth muscle tone have been provided. It is likely that the action is mediated by a shift in the ionic composition of the muscle cell. These findings prompt a search for compounds occurring in the plasma of normals and patients with essential hypertension which, by a similar action, could alter the tone of vascular smooth muscle.

Proposed Course of Project: An attempt will be made to increase the sensitivity of the vessel strip to the known steroids tested and to develop a bioassay procedure for testing the presence of such substances in biological fluids.

5.b

ANALYSIS OF NIH PROGRAM ACTIVITIES

Budget Data Sheet

10. NHI-56
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-56
Serial No.

16. None

17. None

ANALYSIS OF NIH PROGRAM ACTIVITIES

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Cardiovascular Hemodynamics (LABORATORY OR BRANCH)

3. None 4. None 5. NHI-57 (SERIAL NO.)

6. Investigations of the Effects of Alterations of Stroke Volume, Aortic Pressure, and Heart Rate on Left Ventricular Oxygen Consumption and Filling Pressure.
PROJECT TITLE

7. S.J. Sarnoff, M.D. and R.B. Case, M.D.
PRINCIPAL INVESTIGATORS

8. E. Braunwald, M.D., W.N. Stainsby, Sc.D., G.H. Welch, Jr., M.D. and Z. Taylor.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Previous observations by Sarnoff, Case and Berglund have demonstrated that an increase in oxygen consumption accompanies an increase in left ventricular stroke work. It has not been known, however, whether it is the increased work *per se* which increases the O_2 requirement or whether one particular component of the work increment (pressure, stroke volume or rate) is largely responsible. The primary purpose of this study is to determine the effects of changes in left ventricular stroke work produced by alterations of the aortic pressure (at a constant stroke volume and heart rate), and of alterations of stroke volume (at a constant aortic pressure and heart rate) on left ventricular oxygen consumption. Similarly, the effects of changes in heart rate (at a constant cardiac output and aortic pressure) on left ventricle oxygen consumption are being examined. Furthermore, the effects of independent and controlled alterations of aortic pressure, stroke volume and heart rate on mean left atrial (left ventricular filling pressures) are under investigation.

Major Findings: A) A canine preparation has been developed in which heart rate, stroke volume and aortic pressure can be controlled and varied independently. Heart rate is controlled by supraventricular stimulation at a rate above the spontaneous heart rate, or by means of ventricular stimulation in a dog with complete heart block produced by interruption of the bundle of His.

Aortic pressure is determined by leading the left ventricular output through a Starling resistance into a reservoir. Stroke volume is controlled by varying the output of a pump which returns the blood from the reservoir to the arterial system of the dog. Pressures in the right and left atrium, the pulmonary and femoral arteries and the aorta are continuously recorded together with left main coronary artery flow, cardiac output and heart rate. Oxygen content and capacity, CO_2 content, pH, and hematocrit are measured in arterial and coronary sinus blood specimens.

B) On the basis of 12 successful experiments, the following results have been obtained: 1) An increase in left ventricular stroke work produced by an elevation of aortic pressure produces an almost proportional increase in left ventricular oxygen consumption. An increase in left ventricular stroke work of similar magnitude produced by increasing the stroke volume results in only a slight increase in oxygen consumption, and hence a marked rise in myocardial efficiency.

2) At constant aortic pressure and cardiac output a progressive increase in heart rate (above a rate of 90/min.) results in a progressive increase in myocardial oxygen consumption, and hence a reciprocal fall in myocardial efficiency.

3) An increase in left ventricular stroke work produced by an increase in stroke volume requires a greater elevation of left ventricular filling pressures than a similar increase in left ventricular stroke work produced by and elevation of aortic pressure.

4) An apparent inconsistency with Starlings second law of the heart (which relates diastolic fiber length to myocardial oxygen consumption) has been observed repeatedly and consistently. With substantial elevations of filling pressure induced by increasing stroke volume, oxygen consumption rises only slightly. However, with relatively small elevations of filling pressure produced by elevating aortic pressure, oxygen consumption rises markedly.

5) Left ventricular function curves carried out by increasing only the stroke volume (at various aortic pressures) indicate that significantly different ventricular function curves occur at various aortic pressures. The highest ventricular function curves are obtained at mean aortic pressures near 150 mm Hg and lower ventricular function curves are observed with both higher and lower aortic pressures.

6) Ventricular function curves fall progressively with increase in heart rate above 100/min., while aortic pressure and heart rate are held constant.

Proposed Course of Project: These findings, if confirmed by continued investigation, are of great importance to our understanding of fundamental cardiac physiology and it is the intention of this laboratory to devote a major part of its efforts in arriving at the firm solution of these problems. Refinement of both the experimental preparation itself and means by which more detailed biochemical information can be simultaneously obtained are contemplated.

Significance: See paragraph above.

6. b

ANALYSIS OF NIH PROGRAM ACTIVITIES

Budget Data Sheet

10. NHI-57
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards and Publications Sheet

15. NHI-57
SERIAL NO.

16. None

17. None

7. a

ANALYSIS OF NIH PROGRAM ACTIVITIES

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Cardiovascular Hemodynamics — LABORATORY OR BRANCH
3. None
4. None
5. NHI-58 — SERIAL NO.

6. Investigation of the Hemodynamics of Experimental Mitral Insufficiency.
PROJECT TITLE

7. E. Braunwald, M.D. and S.J. Sarnoff, M.D.
PRINCIPAL INVESTIGATORS

8. G.H. Welch, Jr., M.D. and R.B. Case, M.D.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The purpose of this investigation is to study, in the dog, the acute hemodynamic consequences of mitral insufficiency. Secondly, the clinical recognition of mitral insufficiency by the contour of the LA pressure pulse is frequently difficult or impossible. By producing an intervention which can significantly increase the degree of mitral regurgitation, namely, by increasing resistance to left ventricular outflow, it is hoped to significantly alter the LA pressure pulse so that the characteristic pulse contour of mitral insufficiency would become evident.

Major Findings: 1) Acute mitral insufficiency has been produced in seven dogs by introducing a tube through the left atrial appendage into the left ventricle. Blood regurgitates from the left ventricle through the tube which has an opening in the left atrium. This tube can be readily inserted into and withdrawn from the left ventricle.

2) When resistance to left ventricular ejection is increased by means of adjustable aortic constriction, or by means of the administration of a vasoconstrictor (Nor-epinephrine), the contour of the left atrial pressure pulse shows relatively little alteration. However, in the presence of mitral insufficiency the "v" wave and the "y" drop in the left atrial pressure pulse become substantially exaggerated.

3) In the presence of mitral insufficiency there was a progressive fall in stroke volume (Potter electroturbinometer) as aortic constriction was increased. This finding is either absent or minimal in the absence of mitral regurgitation.

4) Recently, a preparation has been developed in which the regurgitant flow may be quantitated. This consists, essentially, of a prosthesis inserted into the apex of the left ventricle through which the blood is led from the left ventricle through a Potter electroturbinometer to a cannula inserted into the left lower pulmonary vein. The degree of mitral insufficiency may thus be regulated by constriction of this by-pass and the hemodynamic effects of known quantities of regurgitant flow are thus studied. It has been demonstrated that with No-epinephrine or aortic constriction, the regurgitant flow is substantially increased.

5) It was interesting to note that the volume of regurgitant flow could be made very large (2 to 4 liters/min.) without producing pulmonary edema levels of left atrial pressure.

Proposed Course of Project: To refine and continue these investigations and apply these techniques to the study of aortic valvular disease as well.

Significance: These studies may lead to a diagnostic test of mitral insufficiency which may be applicable to patients with this disease. They also should provide an understanding of the hemodynamics of this lesion.

7.b

ANALYSIS OF NIH PROGRAM ACTIVITIES

Budget Data Sheet

10. NHI-58
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-58
SERIAL NO.

16. None

17. None

8. a

ANALYSIS OF NIH PROGRAM ACTIVITIES

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Cardiovascular Hemodynamics
LABORATORY OR BRANCH

3. None

4. None

5. NHI-59
SERIAL No.

6. Investigation of the Effect of pH on Coronary Sinus Oxygen Content.
PROJECT TITLE

7. R.B. Case, M.D. and S.J. Sarnoff, M.D.
PRINCIPAL INVESTIGATORS

8. Z. Taylor
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Earlier work of the above has demonstrated a constant myocardial oxygen extraction ratio regardless of hematocrit, work load, or filling pressure. This suggests that a constant pO_2 is maintained in the coronary sinus blood and the myocardium and further that the intrinsic regulation of coronary vascular resistance is aimed at the maintainance of constant myocardial pO_2 levels much as respiratory activity is regulated by the effect of CO_2 or pH on the respiratory center. Proof of this hypothesis will be attempted by (1) calculating pO_2 values over a wide variety of conditions using pH and oxygen saturation in the coronary sinus, and (2) varying pH alone to determine whether oxygen content changes as a result.

Major Findings: A method has been developed for continuous withdrawal of coronary sinus blood. A catheter inserted in an anterior coronary vein is passed to about 2 cm. from the right atrium and tied in place without occluding venous flow. Blood is then passed via a Bowman pump through a continuously recording pH meter and returned to the jugular vein.

The pH of the dog has been varied from 6.6 to 7.7 by means of hyperventilation or addition of CO_2 to the breathing bag of a Jefferson respirator. Variation of the pH over so wide a range has necessitated a determination of the validity of the Rosenthal temperature correction over this range. Also, no oxygen dissociation data are available beyond the range of 7.2 - 7.6. However, from the few dogs thus far studied, the pO_2 of coronary sinus blood seems to remain constant at about 20-25 mm Hg unless coronary insufficiencey supervenes.

Proposed Course of Project: The preparation described above will be followed in a larger series of animals. Also, pH and oxygen saturation data will be taken from the coronary flow and work

experiments being carried on in this laboratory to see if these values fit in with the present hypothesis. The Rosenthal factor must be validated over the wide pH range involved, by simultaneous pH determinations at room temperature and at 37.5° C. If this hypothesis appears to be a correct one from ensuing data, direct pO_2 determinations or an extension of the pH-oxygen saturation data will be necessary.

Significance: If the above hypothesis is correct, we shall have been successful in demonstrating the dominant element in the control of coronary blood flow.

ANALYSIS OF NIH PROGRAM ACTIVITIES

Budget Data Sheet

10. NHI-59
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-59
SERIAL NO.

16. None

17. None

9.a

ANALYSIS OF NIH PROGRAM ACTIVITIES

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Cardiovascular Hemodynamics (LABORATORY OR BRANCH)

3. None 4. None 5. NHI-60 (SERIAL NO.)

6. Rapid Spectrophotometric Determination of Oxygen Content, Capacity and Saturation. (PROJECT TITLE)

7. W.N. Stainsby, Sc.D., Z. Taylor, R.B. Case, M.D. (PRINCIPAL INVESTIGATORS)

8. None

9. PROJECT DESCRIPTION

Objectives: To prepare for use in this laboratory a modification of the Nahas spectrophotometric blood oxygen saturation method of Stainsby, Faler and Lilienthal for the determination of oxygen content on dog blood.

Major Findings: Early comparisons of duplicates analyzed spectrophotometrically and gasometrically showed the spectrophotometric method to be unstable. Use of copper ammonium sulfate solution as a standard for adjusting cuvette thickness shows promise of eliminating this difficulty. Some one-hundred and fifty separate analyses have been completed with duplicates analyzed gasometrically. It is expected that a nomogram can be constructed and the method will be ready in the near future.

Proposed Course of Project: To refine this technique to the point of experimental and clinical applicability.

Significance: The rapid determination of blood oxygen values will greatly amplify the value of a substantial portion of the experimental program of this laboratory and facilitate work in this field.

9.b

ANALYSIS OF NIH PROGRAM ACTIVITIES

Budget Data Sheet

10. NHI-60
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-60
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Gerontology — LABORATORY OR BRANCH
3. — SECTION
4. Baltimore City Hospitals, Baltimore 24, Maryland — LOCATION(IF OTHER THAN BETHESDA
5. NHI-61 C — SERIAL NO

6. Cardiovascular Hemodynamics. I. Arterial performance in man. — PROJECT TITLE

7. Dr. Milton Landowne — PRINCIPAL INVESTIGATOR(S)

8. Flath — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

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 is compared to the theoretical behavior of models of increasing complexity.

Pertinent interarterial 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.

NHI-61C
SERIAL NO.

PROJECT TITLE: Cardiovascular Hemodynamics. I. Arterial performance in man.

9. PROJECT DESCRIPTION: (continued)

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.

Major findings: Agewise changes in brachioradial arterial behavior have been characterized by our method of study as parameters of a descriptive equation.

Differences also have been identified in the behavior of the brachioradial arteries (a) of hypertensive and normotensive subjects at conditions of comparable pressures and age; and (b) of clinically "sclerotic" and "non-sclerotic" subjects of comparable ages.

These differences provide a basis for interpreting the changes with age, in general as indicating reduction in the parameters denoting muscular participation as well as the development of the "sclerotic" pattern in this muscular artery.

A relatively simple model is appropriate to explain the fall of pressure in diastole. It requires that the vessels distal to the major arteries be considered as contributing a significant storage, or elastic function.

Significance to Heart Research: Represents advance in concepts and in knowledge of the physiology of the circulation in human subjects.

Illustrates the role of more basic scientific inquiry in human circulatory physiology.

Proposed course of project: Continuation of recording and analyses of pressure curves under various experimental conditions and at several sites in the arterial tree. Continued study of the influence of reflected components.

Analysis of NIH Program Activities

Project Description Sheet

10. NHI-61C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Baltimore City Hospitals

14. None.

Honors, Awards, and Publications

15. NHI-61C
SERIAL NO.

16. None.

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Gerontology — LABORATORY OR BRANCH
3. — SECTION
4. Baltimore City Hospitals, Baltimore 24, Maryland — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-62C — SERIAL NO.

6. Hemodynamics. II. Cardiac performance in man.
PROJECT TITLE

7. Dr. Milton Landowne
PRINCIPAL INVESTIGATOR(S)

8. J. A. Falzone, Yaffa, Benser
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To study the performance and functional limitations of the living human heart as affected by age and disorders of the circulation.

Methods employed: Studies in sizeable numbers of normal subjects require techniques which are generally acceptable to the subjects. Cardiac output was measured in duplicate by a dye dilution technique, and brachial intra-arterial pressure was measured by needle puncture and resistance strain gage. These methods have been adapted to permit additional observation during exercise, and in relation to concurrent measures of ventilation and respiratory gas exchange.

Sinusoidal force of varying frequency is used to study the body displacement under conditions used in clinical ballistocardiography.

Clinical material: Subjects are obtained from our 60 bed male ward, and from other wards of the hospitals.

Major findings: Interpolation of cardiac output from prediction formulae based on blood pressure curves has been shown to be only approximately valid under steady state conditions of rest, and further difficulties lie in the way of such estimates for non-steady state conditions.

When sinusoidal force is applied to the body over the frequency range used in clinical ballistocardiography, the body does not respond as a unified mass; this invalidates one of the assumptions made in ballistocardiographic work. Individual differences in the response of the body to external forces indicate that dissimilar body motions such as age changes in ballistocardiograms can be due to similar forces.

Analysis of NIH Program Activities

Project Description Sheet

NHI-62C
SERIAL NO.

PROJECT TITLE: Hemodynamics. II. Cardiac performance in man.

9. PROJECT DESCRIPTION: (continued)

Significance to Heart Research: Represents a contribution to the understanding of the function of the heart and the total circulation, and how these are affected by age.

Provides reliable normative information about cardiovascular function in man, and its age trends, for clinical as well as research use.

Enables improved clinical identification of disorders of the heart and aorta.

Proposed course of project: Cardiac output and blood pressure are to be measured under standard conditions of rest and at two levels of exercise in a selected series of subjects.

The response of 2 body sites to an applied sinusoidal force of essentially constant amplitude and over a selected frequency range will be compared for subjects of different age and ballistocardiographic patterns.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-62C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 o5 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Baltimore City Hospitals

14. None.

Honors, Awards, and Publications

15. NHI-62C
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Brandfonbrener, M., M. Landowne and N. W. Shock: Changes in cardiac output with age. Circulation, 12: (4), 557-566, Oct. 1955.

2. Landowne, M., M. Brandfonbrener and N. W. Shock: The relation of age to certain measures of performance of the heart and the circulation. Circulation 12: (4), 567-576, Oct. 1955.

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Gerontology — LABORATORY OR BRANCH
3. — SECTION
4. Baltimore City Hospitals, Baltimore 24, Maryland — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-63C — SERIAL NO.

6. Cardiovascular Hemodynamics. III. The peripheral circulation in man. — PROJECT TITLE

7. Dr. Milton Landowne — PRINCIPAL INVESTIGATOR(S)

8. Pratt - Christ — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

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 controlled 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.

Major findings: Under conditions of skin cooling, the agewise increase in equilibrium toe temperature is not associated with a cooling of "core". Patterns of cooling vary, at least among individuals. The measures of resting and vasodilated flow show considerable variability, the series is yet too small to warrant agewise analysis.

Significance to Heart Research: Describes the peripheral vascular responses of 'normal' adult and older men, providing standards.

Shows how commonly limitation in peripheral vascular function may be encountered among adult males without clinically demonstrable disease.

Represents an aid to the diagnosis of peripheral vascular disease. Provides a critical evaluation of the use and limitations of secondary indices of circulatory function.

Analysis of NIH Program Activities

Project Description Sheet

NHI-63C
SERIAL NO.

PROJECT TITLE: Cardiovascular Hemodynamics. III. The peripheral circulation in man.

9. PROJECT DESCRIPTION: (continued)

Proposed course of project: Skin cooling rate curves will be analyzed to see if the age differences in temperature are related to demonstrable differences in heat regulation.

Proposed course of project: Venous occlusion plethysmography will be used to estimate peripheral circulation under various conditions of 'maximal' vasodilatation. 'Minimal' peripheral resistance and the changes induced in resistance are to be studied in relation to alterations of arterial pressure.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-63C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Baltimore City Hospitals

14. None.

Honors, Awards, and Publications

15. NHI-63C
SERIAL NO.

16. None.

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Gerontology — LABORATORY OR BRANCH
3. — SECTION
4. Baltimore City Hospitals, Baltimore 24, Maryland — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-64 — SERIAL NO.

6. Age changes in renal physiology — PROJECT TITLE

7. N. W. Shock, F. A. Silverstone — PRINCIPAL INVESTIGATOR(S)

8. Yiengst, Barrows, Lewis, Roeder — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objectives of this project are to describe the effects of aging on renal function. Age differences in the ability of the kidney to excrete various substances and to re-adjust blood composition when experimental displacements are induced are under investigation. The effects of age on renal responses to various drug and endocrine products are also being investigated.

Methods employed: Standard clearance methods for estimating GFR, renal plasma flow and T_m are used. In addition, analyses of blood and urine for other substances under study such as vitamin B_{12}, ammonia, acidity, and other electrolytes are carried out under conditions of experimental administration of these substances as well as during acid and alkali loading.

Studies on renal tissue from rats of various ages are also being investigated by the Warburg technique.

Major findings: When the protein intake was increased to 83 gm./day young subjects showed a greater increment in GFR than did old. This difference in GFR response is associated with a greater rise in NPN content on the blood in the old subjects.

Intravenous infusion of acid results in a marked reduction in urine flow in the human. A prompt increase in the output of titratable acid and ammonia occurred in all subjects, even though no change in blood pH could be detected in some of them. It is apparent that the renal response to acid loading is a sensitive mechanism, but in view of the marked alteration in urine flow induced it does not seem that infusion of acid is an appropriate technique to test individual differences in the ability of the kidney to form ammonia.

Analysis of NIH Program Activities

Project Description Sheet

NHI-64
SERIAL NO.

PROJECT TITLE: Age changes in renal physiology.

9. PROJECT DESCRIPTION: (continued)

The endogenous oxygen uptake, the concentration of succinoxidase, succinic dehydrogenase, desoxyribose, ribose nucleic acid, protein nitrogen (but not total nitrogen) were found to decrease 10-15% in the kidney of old (24 month) female rats when based on wet weight. Similar changes were found in male rats. There was also an increase in kidney to body weight ratio with increasing age. Thus aging in the kidney of the rat seems to be related to a loss of protoplasm or an increase in hydration. In male rats the succinoxidase activity decreased although the DNA and RNA content was unchanged. These observations may indicate a change in enzyme activity that precedes the loss of cellular elements.

Significance to Heart Research: The incidence of renal disease increases with age. Consequently it is important to identify changes in renal function that may occur with age prior to the development of clinically identifiable renal disease. Knowledge about age changes in renal mechanisms is necessary for the development of rational methods for the prevention and treatment of kidney disease.

Proposed course of project: Serial determinations on age changes in kidney function in the same subjects will be continued. Experiments to determine alterations in glomerular permeability will also be initiated. The experiments on rats will be extended to investigate other enzyme systems, and to determine the relative importance of cellular loss or increased hydration in the tissue.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-64
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Baltimore City Hospitals

Johns Hopkins University School of Hygiene and Public Health

14. None

Honors, Awards, and Publications

15. NHI-64
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Gerontology — LABORATORY OR BRANCH
3. — SECTION
4. Baltimore City Hospitals, Baltimore 24, Maryland — LOCATION(IF OTHER THAN BETHESDA)
5. NHI-65C — SERIAL NO.

6. Pulmonary Physiology as related to age — PROJECT TITLE

7. A. H. Norris, N. A. Falzone — PRINCIPAL INVESTIGATOR(S)

8. Melvin, Mrs. Benser — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To describe age changes in pulmonary function. These studies involve measurements of the volumes of lung compartments, the functional capacity of the pulmonary system, including mechanical aspects of bellows function and the responsiveness of respiratory function to experimental stimulation and displacement.

Methods employed: In addition to the standard methods of measuring respiratory volumes, a helium washout technique has been developed to give estimates of functional volumes as well. An attempt to relate anatomical measurements made directly on the chest, as well as roentgenographic measurements, including motion of the diaphragm, with functional measurements is being made. In addition, the laboratory measurements are being correlated with clinical estimates of pulmonary function.

Major findings: Measurements of pulmonary function have been made in 135 subjects, aged 20-89 years and the results analyzed statistically. The age reduction in vital capacity is largely a reflection of the overall reduction in body size with age. The important age change is a gradual increase in residual volume at the expense of vital capacity. It has also been found that the reduction in maximum voluntary ventilatory volume in the older ages can be attributed to a reduced increment in the voluntary rate of ventilation and not to a reduction in the tidal volume attained.

Significance to Heart Research: Prevention of the increased incidence of chronic pulmonary disease in elderly people requires knowledge about age changes in pulmonary function. Previous investigations from this laboratory have shown that older subjects increase their respiratory

NHI-65C
SERIAL NO.

PROJECT TITLE: Pulmonary Physiology as related to age

9. PROJECT DESCRIPTION: (continued)

volume much more than do young subjects when a standard exercise is performed, even when the oxygen requirements for the work are the same. Hence studies of pulmonary volume, dead spaces, and rates of transfer of gases across the alveolar membrane are of fundamental interest.

Proposed course of project: Studies of age changes in the elastic properties of the intact lung will be attempted. Since aging is accompanied by a loss of tissue elasticity, measurements on the lung may afford a useful index of physiologic age. In addition, estimates of the work involved in respiration will be made. Studies on the permeability of the alveolar membranes are also under consideration.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-65C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Baltimore City Hospitals

14. None

Honors, Awards, and Publications

15. NHI-65C
SERIAL NO.

16. None.

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute 2. Gerontology
LABORATORY OR BRANCH

3. 4. Baltimore City Hospitals
Baltimore 24, Maryland 5. NHI-66C
SECTION LOCATION(IF OTHER THAN BETHESDA) SERIAL NO.

6. Age changes in metabolism and endocrine function
PROJECT TITLE

7. N. W. Shock, S. P. Baker, G. W. Gaffney, F. A. Silverstone
PRINCIPAL INVESTIGATOR(S)

8. Mrs. Dorcas, Robinson, Griffin
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objectives of this project include a description of age changes in total metabolism, a characterization of the nutritional requirements of aging people and a description of age changes in the functional capacities of various endocrine glands.

Methods employed: For studies on nutritional requirements and the metabolic effects of steroid hormones an eight bed metabolic balance ward, with a special kitchen for food preparation and laboratories for the chemical analysis of foods, urine and stools has been established. Patients from the research ward of the Section (located in the Baltimore City Hospitals) are observed under rigorously controlled conditions for periods of 3-6 months during which time dietary intakes of Ca, N, etc. are systematically varied, or various steroid hormones are administered. Studies of age differences in carbohydrate utilization are carried out by means of the intravenous glucose tolerance test, both with and without the simultaneous administration of known amounts of insulin. Estimates of thyroid function include determinations of basal oxygen consumption by the open circuit method, the rate of uptake of I^{131} by the thyroid gland, and estimates of PBI levels in the blood. Estimates of fluid compartments utilize thiocyanate (for extra cellular fluid volume) and antipyrine (for total body water). A method for determining the fat content of the body from estimates of specific gravity where the body volume is estimated by the displacement of helium is being developed. Studies on lipid metabolism include estimates of the amount of clearing factor released by the administration of small amounts of heparin.

Major findings: Preliminary results indicate that in females there is no systematic reduction in basal oxygen consumption with age when total body water (or intracellular water) is used as an index of body size.

NHI-66 C
SERIAL NO.

PROJECT TITLE: Age changes in metabolism and endocrine function.

9. PROJECT DESCRIPTION: (continued)

The methodology for determining the rate of uptake of I^{131} by the thyroid gland, the rate of disappearance of injected I^{131} from the blood and the rate of excretion in the urine has been set up and standardized.

Major findings: There is a gradual reduction in the basal vitamin B_{12} levels in the plasma between the ages of 50 and 90 years. Whether this is evidence of a relative vitamin B_{12} deficiency in older people remains to be seen.

Using the intravenous glucose tolerance test it has been found that insulin has a significantly greater effect on the rate of disappearance of glucose from the blood in young subjects than in old. It is believed that this is a metabolic difference and is not due simply to a reduction in the amount of functioning protoplasm in the older subjects.

A method, based on the rate of in vitro clearing of standard substrate, has been devised and standardized. Significant individual differences in the amount of clearing factor released after intravenous administration of 3 mg. heparin have been demonstrated.

Additional evidence of a reduction in the amount of functioning tissue has been obtained. Although total body weight shows only a slight reduction with age on the average, there is a significant reduction in lean body weight when calculated from total water content.

Significance of Heart Research: At present the dietary requirements of elderly people are largely inferred from studies made on young adults. With the increasing number of the aged in our population it is essential that we learn something about the basic dietary requirements of the group Many of the phenomena of aging bear a superficial resemblance to endocrine deficiencies. It is important to find out the extent to which the fundamental metabolic processes change with age and the influence of various hormones on these phenomena. It is also important to determine whether the decrements in physiologic function are due to the gradual loss of functioning tissue or whether there is a change in the functional capacity of the tissue remaining.

Proposed course of project: The study of the relationship between basal metabolism and body water content in females will be continued. The investigation of age differences in activity of the thyroid will be continued. One aspect of this study involves testing the responsiveness

Analysis of NIH Program Activities

Project Description Sheet

NHI-66C
SERIAL NO.

PROJECT TITLE: Age changes in metabolism and endocrine function.

9. PROJECT DESCRIPTION: (continued)

of the thyroid gland to exogenous TSH. Studies on individual differences in the release of clearing factor will be continued. It is hoped that the long term effects of the administration of anabolic hormones can be tested, using the metabolic balance technique. An evaluation of the factors involved in age changes in body size and composition will be carried out.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-66C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Baltimore City Hospitals

14. None

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-66C
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Shock, N. W. and M. J. Yiengst: Age changes in basal respiratory measurements and metabolism in males. J. Geront., 10: (1), 31-40, Jan. 1955.

2. Shock, N. W., D. M. Watkin and M. J. Yiengst: Metabolic aspects of ageing. Chap. 4 "Nutritional Aspects of Ageing", In: Report of 3rd Cong. Int. Assoc. Geront., London, 1954, "Old Age in the Modern World". E. & S. Livingstone Ltd., London, 1955, pp. 127-137.

3. Watkin, D. M., J. M. Parsons, M. J. Yiengst and N. W. Shock: Metabolism in the aged; the effect of stanolone on the retnetion of nitrogen, potassium, phosphorus, and calcium and on the urinary excretion of 17-keto, 11-oxy, and 17-hydroxy steroids in eight elderly men on high and low protein diets. J. Geront., 10: (3), 268-287, July 1955.

4. Shock, N. W.: Metabolism and age. (Review). J. chronic Dis., 2: (6), 687-703, Dec. 1955.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

N. W. Shock was appointed to the Research Committee of the Circulation Section of the American Heart Association.

N. W. Shock was appointed to the North American Committee for the First Pan American Gerontological Congress to be held in Mexico City, September 1956.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Gerontology
LABORATORY OR BRANCH

3. _____
SECTION

4. Baltimore City Hospitals
Baltimore 24, Maryland
LOCATION (IF OTHER THAN BETHESDA)

5. NHI-67
SERIAL NO.

6. Age changes in cellular and tissue physiology
PROJECT TITLE

7. N.W. Shock, Barrows, Yiengst
PRINCIPAL INVESTIGATOR(S)

8. Roeder, Starnes, Lewis, Smith
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objectives of this project are to investigate age changes in the chemical composition of tissues and alterations in enzyme activity of tissues, cells and their components. A primary goal of the project is to develop an index of the number of functioning cells that are present in a tissue.

Methods employed: These experiments will be carried out initially on slices and homogenates prepared from various tissues of young and old rats. The Warburg technique will be used for determinations of oxygen uptake. Standard methods of enzyme chemistry will be used for other systems, such as the succinoxidase system. In addition chemical analyses for DNA, RNA, nitrogen, protein, and electrolytes will be made.

Major findings: Studies on the intra and extra cellular components of muscle tissue from old and young rats indicate that aging is accompanied by a decreased concentration of cells per gram of tissue.

With increasing age, the kidney to body weight ratio increased in both sexes, in contrast to the heart to body weight ratio which increased slightly in only the male animals. The liver to body weight ratio was unchanged with age in both sexes. As stated before, the endogenous oxygen uptake as well as the activity of specific enzyme systems of the kidney diminished significantly with age when referred to wet weight. Since the decrement was shared by the intracellular components, it is presumed that the reduction in enzyme activity is largely a reflection of the reduced number of cells in the old female kidney. In contrast, only the succinoxidase activity showed a significant reduction in heart tissue. In the case of liver, no significant age changes were found in any of the enzyme systems tested.

NHI-67
SERIAL NO.

PROJECT TITLE: Age changes in cellular and tissue physiology

9. PROJECT DESCRIPTION: (continued)

Results obtained thus far indicate a differential effect of aging in the three organs studied. The largest changes have been found in the kidney. Changes in only some factors (such as succinoxidase activity) appear in the heart, whereas there were no changes observed in the liver. Since the succinoxidase system plays an important role in energy production, it is possible that the reduction in activity is a fundamental initiating change which occurs in aging and precedes the breakdown of the cell; this is offered only as a working hypothesis.

The methodology for the separation of nuclei has been improved and an apparatus for the production of density gradients in sucrose solutions has been constructed according to the principles described by Anderson. Preliminary trials have indicated that at least two different populations of nuclei can be separated from liver homogenates.

Significance to Heart Research: These studies attempt to examine the aging process at a tissue and cellular level. Since the loss of cells of various tissues is a primary factor in the reduction in reserve capacities of various organ systems that occurs with age, it is important to determine what functional changes occur that prevent individual cells from maintaining their existence. It is also important to know whether these changes are a fundamental characteristic of the cell or whether it is secondary to circulatory impairments in aging animals.

Proposed course of project: Additional enzyme systems will be surveyed for age changes, including estimates of oxidative phosphorylation. In addition studies on age differences in life span and other characteristics of the red blood cell in man. Age differences in the DNA content of nuclei will be investigated. In addition studies on age differences in protein synthesis in the rat will be begun.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-67
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITSOF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Baltimore City Hospitals

Johns Hopkins University School of Hygiene and Public Health

14. None.

Honors, Awards, and Publications

15. NHI-67
SERIAL NO.

16. None.

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Gerontology
LABORATORY OR BRANCH

3.
SECTION

4. Baltimore City Hospitals
Baltimore 24, Maryland
LOCATION(IF OTHER THAN BETHESDA)

5. NHI-68C
SERIAL NO.

6. Age changes in human performance
PROJECT TITLE

7. N. W. Shock, A. H. Norris, J. A. Falzone
PRINCIPAL INVESTIGATOR(S)

8. Manaras, Phillips
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: This project is designed to study the effects of aging on (a) the physiologic responses to exercise (b) the rate of recovery of physiologic equilibrium after exercise (c) muscular efficiency and (d) work output and fatigue. In addition the factors responsible for limitations in performance observed in older people will be evaluated.

Methods employed: Measured amounts of physical work will be obtained in subjects of varying ages by means of a calibrated arm ergometer and a treadmill. Measurements of oxygen uptake, CO_2 production, respiration rate, 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 chemical analysis of 20-25 samples of expired air. Other studies will include measurements of the speed of nerve conduction, reflex delay time, and muscle action potentials. These phenomena will be recorded from micro electrodes and observed on an 8 channel electroencephalograph or on the dual beam oscilloscope as the experiment demands.

Major findings: The responses of 70 subjects, aged 20-90 years, to standardized exercise have been estimated and a preliminary regression analysis has been completed. Measurements of ventilation volume, O_2 uptake, CO_2 elimination, pulse rate and blood pressure were made at 3 levels of work (500 Kg.M. at 135 Kg.M./min., 500 Kg.M. at 267 Kg.M./min. and 350 Kg.M. at 450 Kg.M./min.) in each subject on different days. The regression analysis shows a significant ($p < .001$) increase with age in maximum ventilatory response, total excess ventilatory response and total excess CO_2 elimination at all levels of work. A significant increase with age in total excess oxygen absorbed and consequent decrease in net mechanical efficiency was observed at only the lowest rate of

NHI-680
SERIAL NO.

PROJECT TITLE: Age changes in human performance

9. PROJECT DESCRIPTION: (continued)

work (500 Kg.M. at 135 Kg.M/min). Since body size diminished with age, all values were recalculated on the basis of surface area. The work levels per M^2 increased significantly with age. Correcting the changes in respiratory values for surface area accentuated all age changes. In addition, estimates of total body water were made on 34 of the subjects tested. Calculation of the respiratory responses on the basis of body water content also accentuated the age changes.

Instrumentation has been designed to measure the net mechanical efficiency of exercise involving relatively few muscle groups. The apparatus will record the angular velocities of a rapid back and forth movement of the forearm about the elbow. The mechanical work performed in the movement can be calculated from the records of change in angular velocity and the moment of inertia of the arm. Preliminary experiments have shown that with our technique the excess oxygen required for the motion performed for short periods (1 min.) can be measured. Thus, calculations of net efficiency are possible.

Significance to Heart Research: The effect of age on human performance is of importance both to industry and medicine. With the increasing number of elderly workers in our population industry is concerned with the question of retirement and has expressed a need for objective methods to determine individual retirement. In medicine the question of the degree of activity that can be permitted elderly patients with varying degrees of cardiovascular disease is of practical importance. This program represents an attempt to provide base line data, but also looks to the development of reliable tests that can be applied to large numbers of subjects. In addition, specific knowledge about the effect of age on performance will increase our understanding of aging in the human.

Proposed course of project: The survey of individual differences in responses to standardized amounts of physical exercise will be continued, with the addition of estimates of cardiac output in selected subjects. In addition estimates of maximum work output for short intervals of time will be determined. Studies on the relationship between oxygen uptake and heat production will be carried out in collaboration with the cooperative study of Human Performance sponsored by the Physiology Study Section. Studies of age differences in neuromuscular function will be initiated. These studies will attempt to discover the physiologic locus of the observed slowing in speed of response that occurs with increasing age.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-68C
SERIA NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Baltimore City Hospitals, Naval Medical Research Institute - cooperative project on Human Performance sponsored by the Physiology Study Section.

14. None.

Honors, Awards, and Publications

15. NHI-68C
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Norris, A. H., N. W. Shock and M. J. Yiengst: Age differences in ventilatory and gas exchange responses to graded exercise in males. J. Geront., 10: (2). 145-155, April 1955.

2. Norris, A. H. and N. W. Shock: Age differences in the efficiency of manual exercise in males. Chap. 8 "Experimental Studies of Changes in Performance with Age", In:Report of 3rd Cong. Int. Assoc. Geront., London, 1954, "Old Age in the Modern World". E. & S. Livingstone Ltd., London, 1955, pp. 214-220.

3. Shock, N. W.: Skill and employment. Publ. Hlth. Rep., 70:(9), 851-854, Sept. 1955.

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Laboratory of Chemistry of Natural Products (LABORATORY OR BRANCH)

3. _____ (SECTION) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NHI-69 (SERIAL NO.)

6. New Brain Tissue Substances
PROJECT TITLE

7. L. H. Tallent
PRINCIPAL INVESTIGATOR

8. Mary L. Diethof
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: New biologically important substances in brain tissue.

Objectives: To isolate, purify and characterize certain new biologically important substances that have been discovered in brain tissue.

Methods Employed: Due to the complexity and intractability of the tissue and the minute quantities of the desired substances present, a variety of microchemical techniques have been employed.

Major Findings: The isolation and purification phases of this work are virtually complete and current efforts are directed to securing sufficient material for chemical and biological studies.

Significance to HEART Research: The substances under investigation are members of a family of chemicals known as indoles. Serotonin, a humoral agent affecting mental processes, is also an indole and is formed from tryptophan in the body. The new compounds under study must have some biological relationship to tryptophan metabolism, and their occurrence in the central nervous system is important to both cardiovascular and mental research.

Proposed Course of Project: Studies are planned for the chemical characterization of the new brain indoles. The structure of these compounds will be investigated as rapidly as possible.

Analysis of NIH Program Activities

Budget Data Sheet

10. NII-69
SERIAL NO.

12.
BUDGET ACTIVITY: RESEARCH

13. Dr. John C. Keresztesy (National Institute of Arthritis and Metabolic Diseases)

14. None.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-69
SERIAL NO.

16. None.

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Laboratory of Chemistry of Natural Products — LABORATORY OR BRANCH
3. _____ — SECTION
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-70 — SERIAL NO.

6. Naturally-occurring Hypotensive Agents
PROJECT TITLE

7. William M. Tallent
PRINCIPAL INVESTIGATOR

8. Mary L. Biethof
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Detection, isolation and characterization of naturally-occurring hypotensive substances.

Objectives: The detection, isolation and characterization of natural products of possible biological importance, and the development of new tools for this type of research.

Methods Employed: Newer micro-techniques have been modified and adapted for this work in order to permit detection and characterization of compounds which occur naturally in quantities so small that they could not have been discovered or studied by conventional methods.

Major Findings: The structure of pinidine, a new substance obtained from pine needles, has been determined, and in connection with this, new chemical and instrumental methods have been developed for use in the structural study of natural products. A rapid and simple method for detecting andromedotoxin and related compounds has been developed. Using this technique, it has been possible to ascertain that andromedotoxin is the only hypotensive agent produced by Rhododendra and related plants. Chemical degradation products of andromedotoxin have also been examined, and the potentially important and unusual observation has been made that some of these have hypotensive effects produced by a different biological mechanism than that of the parent substance.

NHI-7C
SERIAL NO.

Significance to HEART Research: New methods for facilitating the important search for new substances for use in hypertension therapy have been developed. In addition, some knowledge regarding the structure of a very active hypotensive agent has been gained, and methods have been developed for aiding characterization studies with other such compounds. These structural studies derive significance from the fact that any new knowledge about the chemistry of hypotensive agents improves the rationale of the search for these drugs, both natural and synthetic.

Proposed Course of Project: Further studies will be concerned primarily with the fact that certain degradation products of andromedotoxin appear to have a hypotensive action that is qualitatively and quantitatively different from that of the parent substance.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIH-7C
SERIAL NO.

12. _____
BUDGET ACTIVITY: RESEARCH

13. Dr. Neil C. Moran (Laboratory of Chemical Pharmacology, NIH-104

14. None.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIH-7C
SERIAL NO.

16. Publications:

Pinus Alkaloids. The Alkaloids of P. sabiniana Dougl. and Related Species. By J. M. Tallent, V. L. Stromberg and E. C. Horning, J. Am. Chem. Soc., 77, 6361 (1955).

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Laboratory of Chemistry of Natural Products (LABORATORY OR BRANCH)

3. _____ (SECTION) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NHI-71 (SERIAL NO.)

6. Structure of Amaryllidaceae Alkaloids
PROJECT TITLE

7. W. C. Wildman
PRINCIPAL INVESTIGATOR

8. C. K. Briggs (in part), P. F. Hignet
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Structure of Amaryllidaceae Alkaloids.

Objectives: The isolation, characterization and determination of structure of new Amaryllidaceae alkaloids is the primary object of this project.

Methods Employed: Isolation and characterization of pure alkaloids from this family were developed along established lines of study. New techniques were devised, as the occasions arose, to more readily determine the structures of these physiologically active materials.

Major Findings: Several new alkaloids of this family have been isolated, characterized and tested physiologically. Structures have been assigned to six alkaloids which have been isolated previously: albomaculine, clivonin, nivaline, neronine, krigeine, and haemanthidine. New synthetic routes to these structures have been examined.

Significance to HEART Research: Since the physiological activity of the alkaloids of this family varies from slight to profound, structural determination of these alkaloids has made possible some correlation between structure and activity. It is hoped that compounds useful in studying hypertension will be found.

Proposed course of project: Research next year will include an intensive search for new, potent hypotensive agents in other families of plants. No new isolation work in the Amaryllidaceae family is anticipated. Nearly completed structural work on some of the Amaryllis alkaloids (crinine, powelline and falcatine) will be concluded.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIH - 71
SERIAL NO.

12.
BUDGET ACTIVITY: RESEARCH

13. Dr. M. C. Moran (Laboratory of Chemical Pharmacology NIH-104, 107)

14. None

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIH-71

16. Publications

Alkaloids of the Amaryllidaceae III. Isolation of Five New Alkaloids from Haemanthus spp. By W. C. Wildman and C. J. Kaufman, J. Am. Chem. Soc. 77, 1248 (1955).

Alkaloids of the Amaryllidaceae IV. Crystalline Alkaloids of Ammocharis Coranica (Ker. Gawl) Herb., Brunsvigia rosea (Lam.) Hannibal and Two Crinum spp. By Lawrence H. Mason, Esther R. Puschett and W. C. Wildman. J. Am. Chem. Soc. 77, 1253 (1955).

Alkaloids of the Amaryllidaceae V. Alkaloids of Nerine falcata Barker and N. laticoma Dur and Schinz. By W. C. Wildman and C. J. Kaufman. J. Am. Chem. Soc., 77, 4907 (1955).

Alkaloids of the Amaryllidaceae VI. The Action of Mild oxidizing Agents on Lycorine and Caranine. By H. M. Fales, E. W. Warnhoff and W. C. Wildman, J. Am. Chem. Soc. 77, 5885 (1955).

On the Structure of Tazettine. By R. J. Highet and W. C. Wildman, Chem. and Ind. 1159 (1955).

Solid Manganese Dioxide as an Oxidizing Agent. By R. J. Highet and W. C. Wildman, J. Am. Chem. Soc. 77, 4399 (1955).

In Press: On the Structure of Haemanthidine. By W. C. Wildman, Chemistry and Industry (1956).

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Laboratory of Chemistry of Natural Products (LABORATORY OR BRANCH)

3. _____ (SECTION) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NHI-72 (SERIAL NO.)

6. New Syntheses of Biologically Important Compounds. (PROJECT TITLE)

7. Gordon N. Walker (PRINCIPAL INVESTIGATOR)

8. None (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Project: New syntheses of biologically important compounds.

Objectives: The objective of this project is to synthesize new heterocyclic compounds, especially indoles, which are significant in mammalian metabolism or in regard to their physiological action, and to develop novel synthetic approaches to various fused-ring compounds related to natural products.

Methods Employed: Ordinary techniques of organic chemistry, catalytic hydrogenation, infrared and ultraviolet spectroscopy.

Major Findings: During the past year emphasis has been placed upon finding improved hydrogenation methods, which are the key to advantageous alterations in many different molecules. Powerful and selective means of reduction have been found and have made possible some new synthetic procedures. A new synthesis of indoles from o-nitrophenylacetonitriles, and a new synthetic approach to fused seven-membered ring compounds (benzsuberenes) involving hydrogenation of certain chalcones derived from dehydroacetic acid, may be considered the most important achievements. In addition, a novel rearrangement of o-nitrophenylacetic acids to acetylanthranils was discovered, and a potentially important new route to 3-substituted indoles was found, starting with the hitherto undescribed formylation of ethyl indole-3-acetate. New syntheses of 3-phenylcyclohexenones and 1-tetralone-2-acetic acids were also investigated successfully.

Significance to HEART Research: Several new methoxyindoles related to tryptamine showed strong, although transient, hypotensive activity in dogs, in contrast with other known tryptamines

which are pressor agents. Recent work has indicated that it will be possible, utilizing new methods, to prepare additional derivatives of tryptamine which may have a lasting effect *in vivo*, as well as some tryptophan metabolites which are involved in biochemical processes affecting the central nervous system. These processes are important in both cardiovascular and mental illness.

Proposed Course of Project: A considerable share of next year's effort will be given over to an exploration of syntheses of potentially valuable indoles related to tryptamine, in an effort to clarify the present knowledge of indole metabolism. Exploration of new synthetic methods for other fused-ring compounds, both homocyclic and heterocyclic, is also projected, special emphasis being placed on novel reductions of enolic and nitrogenous systems.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIH-72
SERIAL NO.

12. _____
BUDGET ACTIVITY: RESEARCH

13. Dr. Neil Moran (Laboratory of Chemical Pharmacology), NIH-104

14. None.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIH-72
SERIAL NO.

16. Publications:

Triton B in Synthesis of 3-Phenylcyclohexenones. By G. N. Walker, J. Am. Chem. Soc., 77, 3664 (1955).

Synthesis of 5,6-Dimethoxyindoles and 5,6-Dimethoxyoxindoles. A New Synthesis of Indoles. By G. N. Walker, J. Am. Chem. Soc., 77, 3844 (1955).

A Novel Rearrangement of o-Nitrophenylacetic Acids. By G. N. Walker, J. Am. Chem. Soc., 77, 6690 (1955).

Hydrogenation of Purpurogallin and Its Derivatives. By G. N. Walker, J. Am. Chem. Soc., 77, 6699 (1955).

In Press:

Synthesis of Dehydropodophyllotoxin Acetate. By G. N. Walker, J. Am. Chem. Soc., 78, 0000 (1956).

Reduction of Enols. New Synthesis of Certain Methoxybenzsuberenes via Reduction of Dehydroacetic Acids. By G. N. Walker. J. Am. Chem. Soc., 78, 0000 (1956).

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Laboratory of Chemistry of Natural Products (LABORATORY OR BRANCH)

3. _____ (SECTION) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NHI-73 (SERIAL NO.)

6. A Fundamental Study of Charge-Transfer Complexes
PROJECT TITLE

7. Warner L. Peticolas
PRINCIPAL INVESTIGATOR

8. None
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Fundamental study of charge-transfer complexes.

Objectives: To study charge-transfer complexes with the aim of obtaining a greater fundamental knowledge of their properties, and to apply this knowledge to metabolic reactions.

Methods Employed: Ultra-violet, visible and infra-red spectra are used to observe and measure the extent of charge-transfer complexes between organic compounds.

Major Findings: The two major findings of the past six months have been (1) charge-transfer forces have been shown to be sufficiently strong to account for some as-yet-unexplained binding of proteins by molecules, (2) carcinogenic hydrocarbons form strong charge-transfer complexes which may be possible intermediates in their carcinogenic action.

Significance to HEART Research: The mechanism of the action of drugs depends to a large extent on the nature of the drug-protein and drug-enzyme interaction. Until all of the forces of interaction between small molecules and proteins are known, it will be difficult to develop chemotherapy along any but empirical lines. The charge-transfer complexes appear to offer possibilities as intermediates in metabolic processes and chemical-protein interaction, and hence an understanding of these complexes and the role they play in biological processes may prove valuable in the understanding and development of chemotherapy in chronic disease, including heart illnesses.

NIH-73
SERIAL NO.

Proposed Course of Project: Quantitative measurements of the equilibrium constants for charge-transfer complexes will continue, both for simple and multiple interaction. Correlations will be sought between the quantitative data and established carcinogenic activity for some polycyclic hydrocarbons. If these correlations are significant, the work will be extended to additional complex-forming compounds.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-73
SERIAL NO.

12. _____
BUDGET ACTIVITY: RESEARCH

13. None.

14. None.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-73
SERIAL NO.

16. None.

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute 2. Laboratory of Chemistry of Natural Products
INSTITUTE LABORATORY OR BRANCH

3. _____ 4. _____ 5. NHI-74 _____
SECTION LOCATION (IF OTHER THAN BETHESDA) SERIAL NO.

6. Chemical and Enzymatic Transformations of Amine Oxides.
PROJECT TITLE

7. C. C. Sweeley
PRINCIPAL INVESTIGATOR

8. _____
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Chemical and Enzymatic Transformations of Amine Oxides.

Objectives: The objective of the project is to establish the chemical pathways of amine oxide reactions and to establish the significance of amine oxides in normal metabolism.

Methods Employed: Chemical reactions of amine oxides are carried out on a microscale and the products are studied by paper chromatographic techniques and various colorimetric procedures. Enzymatic reactions of amine oxides are studied with tissue homogenates and preparations of particulate cell components. Cellular fractionation procedures are employed and the products are studied by micro isolation techniques.

Major Findings: The reactions of an amino acid oxide, N,N-dimethylglycine oxide, were studied in detail. It reacts chemically by a process catalyzed by metal ions. One mode of reaction leads to an oxidative dealkylation whereas a second mode of reaction results in a decarboxylation. Enzymatic studies are in process.

Significance to HEART Research: The chemistry of amine oxides is related to one of the most important of all body processes; the formation and transfer of methyl units through a one-carbon intermediate at the oxidation level of formaldehyde. These compounds are formed readily by the enzymatic oxidation of amines, and a newly-discovered rearrangement reaction is involved in their further reaction. The particular sequence under study is involved in the glycine-choline cycle, and this is of fundamental importance to the functioning of the entire living organism.

NIH-74
SERIAL NO.

Proposed Course of Project: Further studies of the chemical reactions of various amine oxides will be continued in order to establish the generality of the new reactions which have been discovered. A considerable proportion of the project will be devoted to a detailed investigation of enzyme-catalyzed transformations of amines and amine oxides.

Analysis of NIH Program Activities

Budget Data Sheet

10. NMI-74
SERIAL NO.

12. _____
BUDGET ACTIVITY: RESEARCH

13. None.

14. None

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NMI-74
SERIAL NO.

16. Publications:

In Press:

tert Amine Oxide Rearrangements. By H. S. Fish, C. C. Sweeley and E. C. Horning, Chemistry and Industry.

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Laboratory of Chemistry of Natural Products (LABORATORY OR BRANCH)

3. _____ (SECTION) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NHI-75 (SERIAL NO.)

6. Determination of the Structure of Tazettine

7. R. J. Highet
(PRINCIPAL INVESTIGATOR)

8. _____
(OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Project: Determination of the Structure of Tazettine.

Objectives: The primary objective of this study is the elucidation of the structure of tazettine, one of the alkaloids of the Amaryllidaceae. New methods of carrying out structural studies are being developed.

Methods Employed: Tazettine has been converted to simpler molecules by conventional chemical procedures and by new methods developed here during the past year. Procedures for preparing these degradation products by synthesis have been investigated.

Major Findings: Degradative studies have resulted in a proposed structure for tazettine. Manganese dioxide, a useful agent in such studies, has been shown to be capable of oxidizing hemiacetals to lactones and amines to imines. Synthetic methods for the preparation of structures related to tazettine are promising but are not yet complete.

Significance to HEART Research: Alkaloidal materials from the Amaryllidaceae have shown hypotensive action. The preparation of related materials with enhanced activity requires a knowledge of the structures of these natural materials. The information gained and methods developed in elucidating the structure of tazettine will be useful in investigating other materials of natural origin which are significant because of their structure or their physiological activity.

Proposed Course of Project: Last year's degradative studies have provided materials which may be the key to a partial knowledge of the stereochemistry of tazettine. Synthetic approaches to these materials have been initiated, and will be pressed during 1956. Chemical use of manganese dioxide will be studied further.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIH-75
SERIAL NO.

12. _____
BUDGET ACTIVITY: RESEARCH

13. None.

14. None.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIH-75
SERIAL NO.

16. Publications

Solid Manganese Dioxide as an Oxidizing Agent. By E. J. Highet and J. C. Wildman, J. Am. Chem. Soc., 77, 4399 (1955).

On the Structure of Tazettine. By E. J. Highet and J. C. Wildman, Chemistry and Industry, 1159 (1955).

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Laboratory of Chemistry of Natural Products (LABORATORY OR BRANCH)

3. _____ (SECTION) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NHI-76 (SERIAL NO.)

6. Search for Physiologically Active Agents from Plant Sources: Studies of Apocynaceae Alkaloids, Structures of Lunasia Alkaloids, and Isolation and Structure of Ocotea Alkaloids. (PROJECT TITLE)

7. Sidney Mary Goodwin (PRINCIPAL INVESTIGATOR)

8. Ann E. Smith (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Project: The investigation of plant materials for new alkaloids having hypotensive activity.

Objectives: To discover new alkaloids of possible use in therapy, to elucidate their structures by chemical degradation and, ultimately, to synthesize them.

Methods Employed: The organic bases are separated from the rest of the plant constituents by conventional extraction procedures. The methods employed to effect their purification, although varying from plant to plant, usually involve chromatography. Physical data such as absorption spectra in the ultraviolet and infrared regions and optical rotation are obtained for the compounds. On the basis of the analyses, spectra, rotation, and chemical knowledge a detailed study is planned. Usually a large number of attempted degradations are required to afford sufficient evidence to allow for a proposal of the structure of an alkaloid. Proof of the correctness of the proposal is achieved when the alkaloid is synthesized. Oftentimes completely new types of chemical structures are found for the first time in natural products and these often constitute leads for new types of drug structures.

Major Findings: Four plants of the Apocynaceae family were investigated to give nine crystalline alkaloids. One was identified as isoreserpiline, another as β-yohimbine and a third is probably another isomer of yohimbine. The others are of unknown structure. Alkaloids have been isolated on several previous occasions from Lunasia amara (Fam. Rutaceae) and the presence of hypotensive

agents was reported. In the present studies seven crystalline alkaloids were isolated. The first one to be investigated chemically proved to be 2-phenyl-4-methoxyquinoline; Ocotea leucoxylon (Fam. Lauraceae) afforded three alkaloids, one of which was identified as dicentrine (an alkaloid previously found in plants of the family Fumaraceae). Of the other two one possesses an interesting and important physiological activity in that it potentiates the action of sedatives. This is characteristic of the reserpine group of alkaloids, but these compounds are not indoles. Further work is needed to define the action in greater detail.

Significance to HEALT Research: There are drugs such as morphine, quinine and reserpine which are alkaloids and it is possible that new ones of similar importance may be found. As pointed out above, types of structures new to the organic chemist may be found which will enrich our knowledge and stimulate further research leading to the development of new synthetic drugs. A knowledge of the structure of new alkaloids without useful physiological activity may also be valuable in pointing out pathways of amino acid metabolism.

Proposed Course of Project: The projects mentioned above will be continued as far as is possible, depending on the supply of plant material, the alkaloidal content and complexity of the structure of the alkaloid. Contributions leading the way toward greater knowledge of plant alkaloids are expected.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIH-76
SERIAL NO.

12. _____
BUDGET ACTIVITY: RESEARCH

13. Dr. Neil C. Moran (Laboratory of Chemical Pharmacology, NHI-104
NHI-107)

14. None.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIH-76
SERIAL NO.

16. None

17. Travel grant from the National Science Foundation toward defraying expenses involved in attending the 14th International Congress of Pure and Applied Chemistry, July, 1955, at Zurich, Switzerland.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Laboratory of Chemistry of Natural Products (LABORATORY OR BRANCH)

3. _____ (SECTION) 4. _____ (LOCATION (IF OTHER THAN BET.ESDA)) 5. NHI-77 (SERIAL NO.)

6. Isolation, Structural Studies and Synthesis of naturally-occurring plant bases, particularly those having hypotensive action.
PROJECT TITLE

7. J. M. Fales
PRINCIPAL INVESTIGATOR

8. Laura Guiffrida
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Isolation, structural studies and synthesis of naturally-occurring plant bases, particularly those having hypotensive action.

Objectives: Elucidation of the structures of compounds in plant materials whose crude extracts exhibit pharmacological activity. Development of new methods and synthetic pathways necessary to achieve the above objectives.

Methods Employed: Alkaloidal extracts from plants which usually have physiological activity are subjected to chromatography and chemical fractionation. The pure constituents, both new and known, are identified by physical constants and attempts are made to interrelate them by chemical reactions. When a sufficient quantity of pure alkaloid is obtained, its pharmacological properties are investigated.

Major Findings: Narcissus Pseudonarcissus was found to contain galanthamine, lycorenine, galanthine, natalensine, and two new alkaloids called methyl pseudolycorine and lycoridine. Methyl pseudolycorine has been found to be related to the previously identified pseudolycorine and its structure has been ascertained both by degradation and synthesis. Galanthamine has been related to the known alkaloid base IX and the structure of galanthine has been determined in the main. A new spectral method for the identification of two structural units has been found.

Significance to HEART Research: Finding a relation between hypotensive action and chemical structure has been a basic goal of chemotherapy. This requires pure materials of known

structure. One alkaloidal extract (of Narcissus Pseudonarcissus) which possessed hypotensive action has been separated into 6 pure alkaloids, two of which were new. Progress has been made on the elucidation of their structures.

Proposed Course of Project: The structure of natalensine and galanthine will be investigated further and synthetic approaches will be used where practical. Other plant families possessing physiological activity will be investigated. Spectral correlations with chemical structure will be sought, using a high-resolution infrared instrument.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIH-77
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. Dr. Neil C. Moran (Laboratory of Chemical Pharmacology, NHI-11C)

Dr. John Keresztesy (National Institute of Arthritis and Metabolic Diseases)

14. None.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIH-77
SERIAL NO.

16. Publications:

Alkaloids of the Amaryllidaceae VI. The Action of Mild Oxidizing Agents on Lycorine and Caranine. By H. M. Fales, E. W. Warnhoff and J. C. Wildman, J. Am. Chem. Soc., 77, 5885 (1955).

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute 2. Laboratory of Chemistry of Natural Products
INSTITUTE LABORATORY OR BRANCH

3. _____ 4. _____ 5. NHI-78
SECTION LOCATION (IF OTHER THAN BETHESDA SERIAL NO.

6. Structure of Caranine
PROJECT TITLE

7. E. W. Warnhoff
PRINCIPAL INVESTIGATOR

8. Patricia Reynolds
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Structure of Caranine.

Objectives: The characterization and determination of the structure of caranine, an alkaloid of the Amaryllidaceae, is the primary object of this project.

Methods Employed: A method for the isolation of pure caranine from Amaryllis belladonna bulbs has been developed. Established organic chemical techniques have been applied to caranine to determine its structure.

Major Findings: The structure of caranine has been determined. A simple method has been developed for the oxidation of Amaryllidaceae alkaloids, and this may be useful in other studies.

Significance to HEART Research: The determination of the structure of this simplest of the Amaryllidaceae alkaloids has simplified the chemical investigation of other Amaryllidaceae alkaloids with more promising hypotensive action. The structures of this entire group of unusual compounds will soon be established.

Proposed Course of Project: During the next year it is expected that the stereochemistry of caranine will be determined. The investigation of ambelline and other Amaryllidaceae alkaloids will be undertaken, particularly crinamine (the compound with the greatest hypotensive activity of any of the Amaryllidaceae alkaloids investigated so far).

Analysis of NIH Program Activities

Budget Data Sheet

10. NIH-78
SERIAL NO.

12. _____
BUDGET ACTIVITY: RESEARCH

13. None.

14. None.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIH-78
SERIAL NO.

16. Publications:

Alkaloids of the Amaryllidaceae VI. The Action of Mild oxidizing Agents on Lycorine and Caranine. By H. M. Fales, E. W. Warnhoff and W. C. Wildman, J. Am. Chem. Soc. 77, 5085 (1955).

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute 2. Laboratory of Chemistry of Natural Products
INSTITUTE LABORATORY OR BRANCH

3. _____ 4. _____ 5. NHI-79
SECTION LOCATION (IF OTHER THAN BETHESDA) SERIAL NO.

6. Synthetic work on Organic Bases.
PROJECT TITLE

7. Helen A. Lloyd
PRINCIPAL INVESTIGATOR

8. Estelle McCann
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Synthetic work on organic bases.

Objectives: One of the objectives of this project was to study new methods for synthesizing cyclic amides. This part of the project has been completed. Another objective is to study the oxidation of heterocyclic bases to N-oxides as hydroxylamines and to observe the behavior of the products under physiological conditions.

Methods Employed: Various established methods of synthetic organic chemistry are employed to prepare the compounds studied in this project. Paper chromatography is one of the main tools used to follow the reactions and identify the different products which are obtained.

Major Findings: A new rearrangement of 7-membered ring lactams to 6- and 5-membered ring compounds has been observed. These findings have been reported in a publication. The work will now be directed toward the study of the oxidation of the organic bases.

Significance to HEART Research: This project is concerned with the study of products obtained by oxidation of different types of tertiary and secondary amines, and also primarily with the study of the reactions (decomposition, dehydration, rearrangement, etc.) of these oxidation products. Both the formation of oxidation products and their behavior will be studied with emphasis on reactions occurring under conditions similar to those existing in the living plant or animal.

Proposed Course of Project: The heterocyclic bases and their oxidation products will be prepared and their behavior will be studied. These studies are related to oxidation processes occurring normally in plants and animals.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIH-79
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None.

14. None.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIH-79
SERIAL NO.

16. Publications:

Formation of Dihydrocarbostyril-3-acetic Acid and Esters by Rearrangement. By H. A. Lloyd, Louise U. Matternas and E. C. Horning, J. Am. Chem. Soc., 77, 5932 (1955).

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Laboratory of Chemistry of Natural Products (LABORATORY OR BRANCH)

3. _____ (SECTION) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NHI-80 (SERIAL NO.)

6. Isolation and Structure of Ormosia Bases
PROJECT TITLE

7. Helen A. Lloyd
PRINCIPAL INVESTIGATOR

8. Estelle McCann
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Isolation and Structure of Ormosia Bases.

Objectives: The objective of this project is to search for new hypotensive agents derived from plant materials.

Methods Employed: The alkaloids are extracted and isolated from the plant by solvent methods. Paper chromatography and electrophoresis are used to identify the various components. The known techniques of synthetic organic chemistry and degradation procedures are employed in the determination of structure.

Major Findings: A major finding of this project has been the isolation of a potent hypotensive agent from the seeds of Ormosia.

Significance to HEART Research: It was found that one of the alkaloids of Ormosia can be transformed by chemical means into a compound possessing strong hypotensive properties. It is important to study the structure of this precursor and of the related alkaloids of Ormosia in order to determine what type of compounds give rise to these particular properties. Once the structure is determined it might be possible to prepare new hypotensive drugs.

Proposed Course of Project: The investigation of the properties and structure of these bases will be continued.

Analysis of NIH Program Activities

Budget Data Sheet

10, NIH-80
SERIAL NO.

12. _____
BUDGET ACTIVITY: RESEARCH

13. Dr. Neil C. Moran Laboratory of Chemical Pharmacology NHI-11C

14. None.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIH-80
SERIAL NO.

16. None.

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Laboratory of Chemistry of Natural Products — LABORATORY OR BRANCH
3. _____ — SECTION
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-61 — SERIAL NO.

6. Chemical Studies of Bufotenine, N,N-Dimethyltryptamine, N,N-Dimethyltryptophan and their Oxides
PROJECT TITLE

7. M. S. Fish
PRINCIPAL INVESTIGATOR

8. N. Marie Johnson, Esther P. Lawrence
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Chemical Studies of Bufotenine, N,N-Dimethyltryptamine, N,N-Dimethyltryptophan and their Oxides.

Objectives: The object of this project is to study the reactions of these compounds under a variety of chemical conditions resembling physiological conditions and to identify the products.

Methods Employed: Classical chemical techniques are employed where possible. Frequently when only small quantities of material are available, various biochemical techniques may be applied. Purification and identification of products are carried out almost exclusively by paper chromatography and ionophoresis.

Major Findings: Most of these materials are known to occur naturally. This fact, together with the results so far obtained by study of the various reactions, suggests new routes of tryptophan metabolism.

Significance to HEART Research: Many studies have been made in the Heart Institute and elsewhere on serotonin (5-Hydroxy-N,N-dimethyltryptamine) and its role in the circulatory and central nervous systems. Bufotenine and N,N-dimethyltryptamine are close chemical relatives of serotonin and both exert action on the central nervous system. Elaboration of the new tryptophan metabolic pathways suggested by the current project will not only complement the serotonin studies but provide new leads concerned with these related materials.

Proposed Course of Project: It is intended to proceed along these lines with studies of the reactions of these materials. Synthetic work is also in progress to assist in the studies. It is believed that the results obtained here will assist materially in elaboration of new metabolic routes of tryptophan.

Analysis of NIH Program Activities

Budget Data Sheets

10. NIH-81
SERIAL NO.

12. _____
BUDGET ACTIVITY: RESEARCH

13. None

14. None.

Analysis of NIH Program Activities

Honors, Awards and Publications Sheet

15. NIH-81
SERIAL NO.

16. Publications:

In press: tert-Amine Oxide Rearrangements. By M. S. Fish, C. C. Sweeley and E. C. Horning, Chemistry and Industry,

tert-Amine Oxide Rearrangements. N,N-Dimethyltryptamine Oxide. By M. S. Fish, N. M. Johnson and E. C. Horning, J. Am. Chem. Soc. 78, 0000 (1956).

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute 2. Laboratory of Chemistry of Natural Products
INSTITUTE LABORATORY OR BRANCH

3. _____ 4. _____ 5. NIH-C2
SECTION LOCATION (IF OTHER THAN BETHESDA) SERIAL NO.

6. Enzymatic and Metabolic Studies of Bufotenine, N,N-Dimethyltryptamine N,N-Dimethyltryptophan, their oxides and related materials
PROJECT TITLE

7. M. S. Fish
PRINCIPAL INVESTIGATOR

8. N. Marie Johnson, Esther P. Lawrence
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Enzymatic and metabolic studies of Bufotenine, N,N-Dimethyltryptamine, N,N-Dimethyltryptophan, their oxides and related materials.

Objectives: This study is designed to parallel chemical studies on the same materials. Since it has been learned that enzymatic and metabolic products of these compounds are identical to or closely related to the products obtained in many chemical reactions, the study of enzymatic processes and of metabolic pathways are simplified.

Methods Employed: Enzymatic studies are carried out mainly by incubating the different materials with various fractions of liver homogenates with a variety of cofactors and under a variety of conditions. The products are then isolated and identified. The metabolic studies have been concerned so far with examination of the urine of animals and humans receiving one of these compounds.

Major Findings: It has been found that N,N-dimethyltryptamine is converted to the oxide under enzymatic conditions. By varying these conditions 3-indoleacetic acid may be found as the major product. Under other conditions the oxide is partially used up to give a material as yet unidentified. Humans receiving bufotenine excrete 5-hydroxy-3-indoleacetic acid as the principal metabolite. Small quantities of unchanged bufotenine are also found.

Significance to HEALTH Research: This project is designed to parallel the chemical study of these materials. The elaboration of new metabolic sequences can be made only by enzymatic and metabolic studies. Related metabolic products are already known to have effects on the circulatory and the central nervous system.

NHI-02
SERIAL NO.

Proposed Course of Project: The course of the chemical studies will help determine the type of enzymatic and metabolic work to be carried out. It is intended to learn which of these naturally-occurring materials can be utilized by animal enzymatic systems, and the products will be identified for further biological study.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIH-82
SERIAL NO.

12. _____
BUDGET ACTIVITY: RESEARCH

13. None.

14. None.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIH-82
SERIAL NO.

16. Publications:

Oxidative N-dealkylation. By M. S. Fish, N. M. Johnson, E. P. Lawrence and E. C. Horning, Biochem. et Biophys. Acta, 18, 564 (1955).

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute 2. Laboratory of Chemistry of Natural Products
INSTITUTE LABORATORY OR BRANCH

3. _____ 4. _____ 5. NHI-83
SECTION LOCATION (IF OTHER THAN BETHESDA) SERIAL NO.

6. Isolation and Identification of Indoles from Natural Sources
PROJECT TITLE

7. M. E. Fish
PRINCIPAL INVESTIGATOR

8. N. Marie Johnson, Esther P. Lawrence
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Isolation and Identification of Indoles from Natural Sources.

Objectives: This project is designed to provide new leads in the area of tryptophan metabolism and to find new compounds of physiological significance.

Methods Employed: Plant and animal sources which are known or believed to contain indole components related to those under present study are examined. The indoles related to tryptophan and its metabolites are identified, or, if their structure is unknown, studies are instituted for the purpose of learning their identity and their physiological properties.

Major Findings: The seeds of the plant, Piptadenia macrocarpa, were found to contain, beside bufotenine (Stromberg, 1954), bufotenine oxide, N,N-dimethyltryptamine, N,N-dimethyltryptamine oxide and a fifth indole material of unknown structure.

Significance to HEART Research: Bufotenine and N,N-dimethyltryptamine were found to have physiological properties similar to serotonin. The occurrence of these two materials in the same plant, together with their oxides, suggested that they were probably related through a new metabolic sequence. This sequence is of interest to Heart research since closely related materials are already implicated as being important in the circulatory and central nervous systems.

Proposed Course of Project: The findings in this project have brought about initiation of two additional studies (Projects NHI-81 and NHI-82). However, new plant materials are being obtained and additional studies thus far made have been promising. It is expected to utilize animal sources as well.

Analysis of NIH Program Activities

Budget Data Sheet

10. NdI-83
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None.

14. None.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NdI-83
SERIAL NO.

16. Publications:

Piptadenia Alkaloids. Indole bases of P. peregrina (L.) Benth. and Related Species. By H. S. Fish, N. H. Johnson and E. C. Horning, J. Am. Chem. Soc., 77, 5892 (1955).

17. None.

Analysis of NIH PROGRAM ACTIVITIES

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Laboratory of Chemistry of Natural Products — LABORATORY OR BRANCH
3. _____ — SECTION
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-83 (1) — SERIAL NO.

6. Screening and Isolation of Naturally-occurring Substances with Hypotensive Action
PROJECT TITLE

7. D. L. Rogerson
PRINCIPAL INVESTIGATOR

8. J. Link, J. Miles, (G. Latimer in part)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Selected plant samples (known to have physiological activity, or from plant families where activity may occur) are screened for alkaloid content or for physiological activity. In cases where the preliminary results are positive, the agents are isolated for study. Observations of hypotensive action are followed by chemical and physiological work.

Objectives: To discover new substances with hypotensive action, and to isolate these materials for chemical and physiological study.

Methods Employed: Established procedures and specialized methods developed in this Laboratory are employed for preliminary screening. Isolation procedures are usually variations of solvent methods and each case usually requires some degree of special attention. Physiological studies are carried out in the Laboratory of Chemical Pharmacology.

Major Findings: Several hypotensive agents have been isolated and supplied for chemical and physiological studies. Continued work on andromedotoxin has given a new hypotensive agent which is described elsewhere. Ormosia preparations have been carried through screening and isolation stages. A number of new observations on potential hypotensive agents have been made, and these will be evaluated as rapidly as possible.

Significance to HEART Research: Studies on physiological mechanisms involved in the control of blood pressure will contribute to an understanding of the failures involved in hypertension, and they are also needed to aid in the development of new drugs. These control mechanisms are best studied through the use of new compounds which affect

the circulatory system. Control mechanisms which are not now recognized may also be found by these studies.

Proposed Course of Project: Current studies will be supported by isolation work as required. New observations made during last year will be explored further, and the approximate level of screening now in operation will be continued through the year.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-83 (1)
SERIAL NO.

12. _____
BUDGET ACTIVITY: RESEARCH

13. Dr. N. C. Moran (Laboratory of Chemical Pharmacology, NHI-104, 107)

Mr. C. O. Erlanson, Section of Plant Introduction, Horticulture Crops, Plant Industry Station, U. S. Department of Agriculture, Beltsville, Md.

Dr. John C. Keresztesy, Section on Fractionation and Isolation, Laboratory of Biochemistry and Nutrition, NIAMD

14. None.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-83 (1)
SERIAL NO.

16. Publications: The work of this unit is incorporated in publications from other areas of this Laboratory.

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Laboratory of Chemistry of Natural Products (LABORATORY OR BRANCH)

3. _____ (SECTION) 4. _____ (LOCATION (IF OTHER THAN BETHESDA)) 5. NHI-83 (2) (SERIAL NO.)

6. Isolation of Components from Tissue
PROJECT TITLE

7. D. L. Rogerson
PRINCIPAL INVESTIGATOR

8. J. Link, J. Miles (G. Latimer in part)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: The isolation of new physiologically significant components from animal or plant tissue.

Objectives: To isolate new substances from plant or animal tissues.

Methods Employed: The chief problem in this work is to isolate materials which are generally present in very small quantities. It is necessary to employ relatively large amounts of tissue, and to use all known methods of isolation, including solvent extractions, ion exchange columns and chromatography. Assay procedures are usually needed to guide the isolation processes.

Major Findings: The most important development during the year was the isolation of four new components from brain tissue. These materials are apparently derived from tryptophan, but are present in very small quantity in animal brain tissue.

Significance to HEART Research: Current physiological studies indicate that tryptophan metabolites, particularly serotonin, may have an important role in governing biological processes in the central nervous system. Effects on the circulation are involved as well. The study of indole alkaloids, in another project of this Laboratory, led to the belief that these plant substances or their derivatives would be present in mammalian metabolism. The present isolation work is directed to securing an adequate supply of material for chemical and physiological studies.

Proposed Course of Project: The present extraction processes will be developed to give higher yields of isolated materials. The accumulation of sufficient pure compounds for further study is an immediate objective of the brain tissue problem. Other tissues of plant or animal origin may be studied.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIH-83(2)
SERIAL NO.

12. _____
BUDGET ACTIVITY: Research

13. Dr. J. C. Keresztesy, Section on Fractionation and Isolation, Laboratory of Biochemistry and Nutrition, NIAMD.

Mr. C. O. Erlanson, Section of Plant Introduction, Horticulture Crops, Plant Industry Station, U.S. Department of Agriculture, Beltsville, Md.

14. None.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NIH-83(2)
SERIAL NO.

16. Publications: The work of this unit is incorporated in publications from other areas of this Laboratory.

17. None.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology
LABORATORY OR BRANCH

3. _____
SECTION

4. _____
LOCATION (IF OTHER THAN BETHESDA)

5. NHI-84C
SERIAL NO.

6. Implication of Serotonin in the Central Actions of Reserpine
PROJECT TITLE

7. Bernard B. Brodie and Sidney Udenfriend
PRINCIPLE INVESTIGATORS

8. Donald F. Bogdanski and Quentin J. Deming
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine which actions of reserpine are mediated through serotonin and indirectly, therefore, to determine the normal functions of serotonin.

Methods employed: Measurement of various physiologic changes after drug administration.

Major Findings: The hypotensive and hypothermic effects following reserpine administration persist for more than 48 hours even though the reserpine has long since disappeared from the body. The return to normal parallels the repletion of the body serotonin depots.

Significance to HEART Research: Provides basic information concerning the humoral agent serotonin.

Proposed course of project: Reserpine, 5-hydroxytryptophan, Marsilid and lysergic acid diethylamide will be investigated to relate many of their central actions to serotonin.

(5)

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-84C
SERIAL NO.

12. Research - Budget Activity

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.

Dr. Quentin J. Deming, Columbia University Research Service, Goldwater Memorial Hospital.

14. None

Honors, Awards, and Publications Sheet

15. NHI-84C
SERIAL NO.

16. None

17. None

5. a

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Chemical Pharmacology — LABORATORY OR BRANCH
3.
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-85C — SERIAL NO.

6. Clinical Biochemical Studies on Patients Recoiving Reserpine — PROJECT TITLE

7. Bernard B. Brodie and P. A. Shore — PRINCIPAL INVESTIGATOR(S)

8. L. J. Haverback, Edward Tomich, R. Kuntzman, T. Dutcher and G. Brecher — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine the effect of reserpine on the serotonin stores in humans and to relate this effect to possible function of serotonin.

Methods Employed: Various clinical tests are made on patients receiving reserpine.

Major Findings: The platelet serotonin levels of several patients have been determined before and after treatment with reserpine (1 mg./day for several days). It has been found uniformly that reserpine causes almost a complete disappearance of serotonin. After reserpine is withdrawn, the serotonin levels return slowly to normal. Hemotological studies on these patients have shown that bleeding time, clotting time, and clot retraction time remain within normal limits even when serotonin has disappeared. Thus, it seems that serotonin plays no role in these processes.

Significance to HEART Research: Provides basic information concerning the humoral agent serotonin.

Proposed course of project: A few more patients will be given reserpine and blood serotonin levels will be run. An effect on other body functions, water and mineral metabolism, blood pressure, etc. will be run in an attempt to find other consequences of reserpine administration.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-85C
SERIAL NO.

11. BUDGET ACTIVITY: RESEARCH

13. IDENTITY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN CALENDAR 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (MAX. 1)

Dr. A. J. Haverback, General Medicine and Experimental Therapeutics Branch

Dr. A. Butcher and Dr. J. Brecher, Clinical Pathology Department, Clinical Center

Dr. Edwin Tomich, Visiting Scientist from Glaxo Laboratories, Hayes, England

14. None

Honors, Awards, and Publications Sheet

15. NHI-85C
SERIAL NO

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Chemical Pharmacology — LABORATORY OR BRANCH
3.
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-86 — SERIAL NO.
6. Role of Serotonin in Brain — PROJECT TITLE
7. Bernard B. Brodie and Parkhurst A. Shore — PRINCIPAL INVESTIGATOR(S)
8. A. Pletscher, E. G. Tomich and R. Kuntzman — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION

Objectives: To apply reserpine and other serotonin-releasing agents as tools in elucidating role of serotonin in the brain.

Methods Employed: Effects of various drugs on body serotonin stores of animals are studied. The effect on animals of loss of serotonin is also studied.

Major Findings: Of the many compounds tested *in vivo* for serotonin releasing activity, only Rauwolfia alkaloids, and of these only the "tranquillizing" alkaloids (reserpine, rescinnamine, 11-desmethoxy-reserpine) effect serotonin release. Small daily doses of reserpine (0.015 mg/kg) in rabbits cause a cumulative effect on brain serotonin levels, with a steady state being finally reached. Reserpine enters and leaves the brain very rapidly. However, the pharmacologic effects (sedation, miosis, etc.) and the alteration of serotonin levels persist long after reserpine has disappeared from the brain. This further indicates that serotonin is the mediator of the pharmacologic effects of reserpine. Pretreatment of rabbits with marsilid, an amine oxidase inhibitor, causes a subsequent dose of reserpine to exert pharmacologic effects identical to those of LSD (excitement, mydriasis, piloerection). Analysis of the brains of these animals showed an almost normal serotonin concentration. This serotonin, however, is presumed to be in a free form, indicating that free serotonin in high concentration acts like LSD.

Significance to HEART Research: This study is of significance in two aspects: (1) Understanding of the nature of the action of reserpine, a drug used in hypertension and also for emotional imbalance, and (2) understanding of the role of serotonin, a substance which is implicated in many body functions including control of blood pressure.

NHI-86
SERIAL NO.

Proposed course of project: Further studies will be made on the results of serotonin imbalance. A search will be continued for other drugs which affect serotonin.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-86
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Dr. A. Pletscher, Visiting Scientist, Hoffmann-La Roche, Switzerland

Mr. E. G. Tomich, Visiting Scientist, Glaxo Laboratories, England

14. None

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-86
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Parkhurst A. Shore, Stanley L. Silver, and Bernard B. Brodie. Interaction of Reserpine, Serotonin, and Lysergic Acid Diethylamide in Brain. Science, Vol. 122, No. 3163, 284-285, August 12, 1955.

Bernard B. Brodie, Alfred Pletscher, and Parkhurst A. Shore. Evidence that Serotonin has a Role in Brain Function. Science, Vol. 122, No. 3177, 968, Nov. 18, 1955.

Parkhurst A. Shore, S. L. Silver, and Bernard B. Brodie. Interaction of Serotonin and Lysergic Acid Diethylamide (LSD) in the Central Nervous System. Experientia, Vol. XI, No. 7, 272, 1955.

Bernard B. Brodie, Parkhurst A. Shore, and S. L. Silver. Potentiating Action of Chlorpromazine and Reserpine. Nature, Vol. 175, 1133, June 25, 1955.

Alfred Pletscher, Parkhurst A. Shore, and Bernard B. Brodie. Serotonin as a Mediator of Reserpine Action in Brain. Journal of Pharmacology and Experimental Therapeutics (in press).

Donald F. Bogdanski, Bernard B. Brodie, Alfred Pletscher, and Sidney Udenfriend. Identification and Assay of Serotonin in Brain. Journal of Pharmacology and Experimental Therapeutics (in press).

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Chemical Pharmacology (LABORATORY OR BRANCH)

3. 4. (LOCATION (IF OTHER THAN BETHESDA)) 5. NHI-87 (SERIAL NO.)

6. Studies on the Mechanism of Serotonin Release by Reserpine

7. Bernard B. Brodie (PRINCIPAL INVESTIGATOR(S))

8. P. A. Shore and Arvid Carlsson (OTHER INVESTIGATORS)

9. PROJECT DESCRIPTION

Objectives: To study the mechanism whereby reserpine causes the release of serotonin.

Methods Employed: Rabbit blood platelets suspended in plasma are incubated with reserpine and other substances. Platelets are then removed by centrifugation and the supernatant analyzed for serotonin.

Major Findings: By employing a suspension of rabbit platelets in plasma as a model system, it has been possible to demonstrate a release of serotonin in vitro from the platelets. It has been found that (1) serotonin releasing activity in vitro as in vivo is restricted to Rauwolfia alkaloids, and specifically, to those which possess sedating ability, (2) no uptake of reserpine by the platelets occurred, indicating that a simple replacement of reserpine for serotonin had not occurred, (3) a general change in permeability had not occurred since the histamine of the platelets was not released by reserpine, (4) no release of serotonin occurred when the system was incubated at 0°C instead of 37°C. This indicates that a chemical process is involved.

Significance to HEART Research: This study is of significance in two aspects: (1) Understanding the nature of the action of reserpine, a drug used in hypertension and also for emotional imbalance; (2) understanding the role of serotonin, a substance which is implicated in many body functions including control of blood pressure.

Proposed course of project: Studies will be continued on the release of serotonin from platelets in vitro in an attempt to find the nature of the serotonin binding within the cell. Attempts will be made to duplicate some of the platelet observations on brain preparations.

Analysis of NIH Program Activities

Budget Data Sheet

10. MH-87
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. MH-87
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Alfred Pletscher, Parkhurst A. Shore, and Bernard B. Brodie. Serotonin Release as a Possible Mechanism of Reserpine Action. Science, Vol. 122, No. 3165, 374-375, August 26, 1955.

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Chemical Pharmacology — LABORATORY OR BRANCH
3.
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-88 — SERIAL NO.

6. Biogenesis and Metabolism of Serotonin — PROJECT TITLE

7. Sidney Udenfriend — PRINCIPAL INVESTIGATOR(S)

8. Donald Bogdanski, Chozo Mitoma and Herbert Weissbach — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To investigate the catalysts and intermediates involved in the formation and metabolism of serotonin; to determine the effects of intermediates and drugs on their metabolic routes and relate them to the observed actions of serotonin.

Methods Employed: Isotopic and chromatographic procedures, enzymatic techniques.

Major Findings: The knowledge gained from the biochemical studies of serotonin has been put to use in elucidating its role in the body. Thus it has been shown that the precursor, 5-hydroxy-tryptophan (5HTP), when administered to animals enters the brain and is there decarboxylated to serotonin. When brain serotonin is increased in this manner (5 - 20 fold) the observed effects resemble those seen after administration of the hallucinogenic agent, lysergic acid diethylamide. In addition, inhibitors of serotonin oxidase result in increased levels of serotonin in brain; this also leads to central disturbance. In pyridoxine deficiency serotonin levels are markedly diminished.

Significance to HEART Research: Provides basic information concerning the humoral agent serotonin.

Proposed course of project: Attempts are being made to demonstrate the conversion of tryptophan to 5HTP using C^{14} tryptophan and carrier 5HTP, in vivo. Studies with 5HTP in vivo are in progress to determine the physiologic disposition, its conversion to serotonin and its pharmacological effects. After appropriate

toxicity studies 5HTP will be tested on patients. Further studies on monoamine oxidase inhibitors are in progress, to find new compounds and to study their effects in vivo, on endogenous and exogenous serotonin. Studies on binding of serotonin and other bases by platelets will be continued. Investigations on the intracellular localization of serotonin in brain will be resumed.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-88
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Dr. Albert Sjoerdsma, General Medicine and Experimental Therapeutics Branch

14. None

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-88
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Sidney Udenfriend, C. T. Clark, and Herbert Weissbach, The Estimation of 5-Hydroxytryptamine (Serotonin) in Biological Tissues, Journal of Biological Chemistry, Vol. 215, No. 1, July 1955.

Sidney Udenfriend, Elwood O. Titus and Herbert Weissbach, The Identification of 5-hydroxy-3-indole acetic Acid in Normal Urine and a Method for its Assay, Journal of Biological Chemistry, Vol. 216, No. 2, October 1955.

Albert Sjoerdsma, Thomas E. Smith, Thomas D. Stevenson and Sidney Udenfriend, The Metabolism of 5-Hydroxytryptamine (Serotonin) by Monoamine Oxidase, Proc. of Soc. for Exp. Biol. and Med., Vol. 89, pages 36-38, 1955.

Sidney Udenfriend, Donald F. Bogdanski, and Herbert Weissbach, Fluorescence Characteristics of 5-Hydroxytryptamine (Serotonin), Science, Vol. 122, No. 3177, pages 972-973, Nov. 18, 1955.

Sidney Udenfriend, Elwood O. Titus, Herbert Weissbach, and Ralph E. Peterson, Biogenesis and Metabolism of 5-Hydroxyindole Compounds, Journal of Biological Chemistry, in press.

Chozo Mitoma, Herbert Weissbach, and Sidney Udenfriend, Formation of 5-Hydroxytryptophan from Tryptophan by Chromobacterium violaceum, Nature, Vol. 175, page 994, June 4, 1955.

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Chemical Pharmacology — LABORATORY OR BRANCH
3.
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-89 — SERIAL NO.
6. Studies on Binding and Intracellular Localization of Serotonin — PROJECT TITLE
7. Donald F. Bogdanski and Herbert Weissbach — PRINCIPAL INVESTIGATOR(S)
8. — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION

Objectives: To determine where serotonin is localized within cells and how it is taken up by blood platelets.

Methods Employed: In vitro incubations with blood platelets and tissue homogenates.

Major Findings: Platelets, in vitro, take up large amounts of serotonin from solution. When serotonin is administered intravenously it is also taken up by platelets. Platelets also bind other organic bases: epinephrine, bufotenine, etc. Preliminary studies on homogenates of brain indicate that a large proportion of serotonin sediments with the fraction containing mitochondria and nuclei.

Significance to HEART Research: Provides basic information concerning the humoral agent serotonin.

Proposed course of project: Studies on the binding of serotonin and other organic bases by blood platelets will be continued. Investigations on the intracellular localization of serotonin in brain will also be continued.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-89
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-89
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Chemical Pharmacology — LABORATORY OR BRANCH
3.
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-90 — SERIAL NO.
6. Platelet Serotonin — PROJECT TITLE
7. Sidney Udenfriend — PRINCIPAL INVESTIGATOR(S)
8. Collette Robillard and Murray Weiner — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION

Objectives: To determine the levels of circulating serotonin in health and in various pathologic states including those involving platelet anomalies.

Methods Employed: Hematologic and chemical techniques.

Major Findings: The normal range of serotonin in human blood is 0.1 - 0.3 μg/ml of blood or 0.4 - 1.4 μg/10^9 platelets. Platelet serotonin levels were normal in a large variety of disease states including hypertension. Platelets markedly reduced in serotonin content were found to behave normally in their influence on coagulation, clot retraction and bleeding time. Conversely platelets (from certain thrombasthenic patients) which had no clot retraction activity, were found to contain the normal amount of serotonin.

Significance to HEART Research: Provides basic information concerning the humoral agent serotonin.

Proposed course of project: This aspect of the serotonin studies has been completed.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-90
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Dr. Murray Weiner, New York University, Goldwater Memorial Hospital, New York, N. Y.

Miss Collette Robillard, General Medicine and Experimental Therapeutics Branch

14. None

Honors, Awards, and Publications Sheet

15. NHI-90
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Murray Weiner and Sidney Udenfriend, Studies on Platelet Serotonin in Man, Circulation (in preparation).

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Chemical Pharmacology — LABORATORY OR BRANCH
3.
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-910 — SERIAL NO.

6. Tryptophan and Serotonin in Malignant Carcinoid — PROJECT TITLE

7. Sidney Udenfriend — PRINCIPAL INVESTIGATOR(S)

8. Herbert Weissbach — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To study serotonin effects in patients with malignant carcinoid.

Methods Employed: In vivo clinical and animal experiments.

Major Findings: A total of five patients have been studied at the Clinical Center; urine samples have been obtained from an additional 6 patients. A biopsy sample of carcinoid tumor was analyzed and found to contain about 1 mg. of serotonin per gram of tumor and several other 5-hydroxyindoles. It also contained 5HTP decarboxylase and serotonin oxidase. When C^{14} 5HTP was administered to carcinoid patients, urinary 5HIAA was highly labelled. The fasting tryptophan blood levels in 2 carcinoid patients were found to be markedly below normal values.

Significance to HEART Research: Provides basic information concerning the humoral agent serotonin.

Proposed course of project: To determine the following: If serotonin is constantly secreted into the blood from the tumor and if there is a rise in serotonin with a flushing episode. If fasting tryptophan levels are always lowered in such patients and if N-methyl nicotinamide excretion is also lowered. The turnover rate urinary 5HIAA and of the tumor serotonin. Also the size of the tumor serotonin pool and of the tumor mass. The damage to the right side of the heart seen in carcinoid patients may be caused by the direct action of serotonin on the heart tissue. Since 5HTP, when administered, enters the heart tissue it will be given as a source of 5HTP to rats and puppies to see whether comparable heart lesions can be produced.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-91C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Dr. Albert Sjoerdsma, Dr. Philip Waalkes, Dr. John Davidson, and Miss Collette Robillard, General Medicine and Experimental Therapeutics Branch.

14. None

Honors, Awards, and Publications Sheet

15. NHI-91C
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Albert Sjoerdsma, Herbert Weissbach, and Sidney Udenfriend. A Simple Test for Diagnosis of Metastatic Carcinoid (Argentaffinoma), J. Am. Med. Assoc. Vol. 159: 397, 1955.

Sidney Udenfriend, Herbert Weissbach, and Albert Sjoerdsma. Studies on Tryptophan and Serotonin in Patients with Malignant Carcinoid, Science (in press).

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Chemical Pharmacology — LABORATORY OR BRANCH
3.
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-92 — SERIAL NO.

6. Physiologic Disposition and Fate of Reserpine — PROJECT TITLE

7. Sidney M. Hess and Bernard B. Brodie — PRINCIPAL INVESTIGATOR(S)

8. Dominick Tocco — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Reserpine is of considerable importance in medicine as a tranquillizing agent and as a hypotensive agent. Its use has raised a number of interesting and important problems. It is the purpose of this study to determine the physiologic disposition and fate of administered reserpine. For this purpose a sensitive and specific method of analysis must be developed.

Methods Employed: A chemical method of analysis has been developed for this problem. Rabbits, dogs, mice, and rats are used.

Major Findings: The fluorometric method for reserpine, previously reported, has been perfected and its sensitivity extended to detect the presence of as little as 0.05 γ/gm of tissue. The similarity of the pKa values and partition ratios of apparent reserpine in tissues, after its administration, with those of authentic reserpine indicate the specificity of the method. The alkaloid enters the rabbit brain in high concentration almost immediately after intravenous administration, its concentration there as early as one minute after administration being higher than the concentration in the plasma and remaining higher for about 45-60 minutes. After 12-13 hours the values are too low to be measured. Other rabbit tissues which were found to be relatively high in reserpine were the lung, kidney and spleen. Of all the tissues, however, only the lung remained high and only this organ and spleen continued to show the presence of reserpine 48 hours after administration. Preliminary observations indicate that in rats reserpine behaves in the same manner with respect to the blood-brain barrier.

NHI-92
SERIAL NO.

Significance to HEART Research: The elucidation of the action of this hypotensive and tranquillizing drug improves our ability to understand and treat the hypertension problem.

Proposed course of project: Studies on the absorption, distribution, excretion, and metabolism of reserpine in experimental animals are continuing. Attempts will also be made to determine whether reserpine is localized within any particular portion of the brain. Efforts to isolate the metabolic products of reserpine are also under way using paper chromatography and solvent extraction. The tissue catalysts responsible for this metabolism will be investigated.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-92
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-92
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Chemical Pharmacology — LABORATORY OR BRANCH
3.
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-93 — SERIAL NO.

6. Isolation of Cardiotonic Substances from Mammalian Tissues — PROJECT TITLE

7. Elwood Titus — PRINCIPAL INVESTIGATOR(S)

8. Stephen Hajdu and Herbert Weiss — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The isolation and characterization of a cardiotonic substance occurring in animal tissues.

Methods Employed: Counter-current distribution, column and paper chromatography for isolation. Bioassays using effects on the Bowditch staircase phenomenon in the isolated frog ventricle.

Major Findings: Cardiotonic material observed in blood and liver of mammals has been found in heart and in adrenal medulla in close association with unidentified choline containing lipids. Although biologically very similar to digitalis steroids, its chemical properties are not typical of these steroids.

Significance to HEART Research: The normally occurring cardiotonic substance in mammalian tissue may be a hormone required for the control of heart action.

Proposed course of project: Physical isolation of the cardiotonic material will be attempted and its pharmacology studied in animals and man. Biosynthesis of the material will be studied.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-93
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-93
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Chemical Pharmacology — LABORATORY OR BRANCH
3.
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-94 — SERIAL NO.

6. Studies on the Biosynthesis of Cardiotonic Steroids — PROJECT TITLE

7. Elwood O. Titus — PRINCIPAL INVESTIGATOR(S)

8. Elliott Schiffmann — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: A cardiotonic steroid produced in the toad represents one of the few examples of digitalis-like compounds occurring in animals, and as such it was of interest to study its biosynthesis.

Methods Employed: Counter-current distribution, column and paper chromatography of steroids produced from radioactively labelled precursors.

Major Findings: The cardiotonic substance from toads is produced from cholesterol, apparently by an unknown metabolic pathway entirely independent of that leading to the bile acids.

Significance to HEART Research: It is hoped that this project will reveal information about the origin in nature of the cardiotonic steroids.

Proposed course of project: Cholesterol suitably labelled with deuterium is being prepared in order to test the hypothesis that biosynthesis of cardiac steroids proceeds by a new pathway of cholesterol metabolism.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-94
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-94
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Chemical Pharmacology — LABORATORY OR BRANCH
3.
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-95 — SERIAL NO.

6. The Preparation of Compounds with Anti-Digitalis Activity — PROJECT TITLE

7. Ellwood O. Titus — PRINCIPAL INVESTIGATOR(S)

8. Anne W. Murray — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Some biological evidence suggests that cardiac lactones hydroxylated in the 17 position might act as antagonists to digitalis-like steroids. An effort is being made to prepare these.

Methods Employed: Microbiological hydroxylation of known steroids. Identification of products by conventional chemical degradation.

Major Findings: Certain molds can hydroxylate and oxidize cardiotonic lactones in several positions. Evidence for the presence of 17-hydroxy steroids among the products has been obtained.

Significance to HEART Research: Although no compounds are known specifically to antagonize the effects of digitalis on heart muscle, such substances would be of interest in theoretical studies of the mechanism of action of the cardiac steroids. They should be of use in both the diagnosis and treatment of digitalis intoxication.

Proposed course of project: Identification of the microbiologically prepared material will be completed. The products will be tested pharmacologically.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-95
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-95
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Chemical Pharmacology — LABORATORY OR BRANCH
3.
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-96 — SERIAL NO.

6. The Biosynthesis and Metabolism of Epinephrine and Norepinephrine
PROJECT TITLE

7. Sidney Udenfriend
PRINCIPAL INVESTIGATOR(S)

8. Lemuell Leeper
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To elucidate the mechanisms of the biosynthesis of epinephrine and norepinephrine by characterizing the intermediate precursors and the enzyme systems involved.

Methods Employed: Standard radioactive techniques.

Major Findings: C^{14} - labelled 3-4 dihydroxyphenylethylamine (DOPAmine) was synthesized. When administered to rats it produced labelling of adrenal epinephrine. The amount of labelling was greater than that obtained with all other labelled precursors. Perfusion of calf adrenal glands with non-radioactive tyrosine and DOPAmine resulted in little, if any, conversion to epinephrine or norepinephrine. When norepinephrine was perfused no detectable increase in epinephrine occurred.

Significance to HEART Research: Epinephrine and norepinephrine are humoral agents involved in maintaining normal tone of the cardiovascular system.

Proposed course of project: Metabolism of tyrosine and catechol compounds will be studied in pheochromocytoma patients in vivo. When available, pheochromocytoma tumors will be studied enzymatically, in relation to catechol formation. Metabolism of epinephrine by tumor tissue and by various animal tissue will be investigated.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-96
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Dr. Albert Sjoerdsma and Mr. William King, General Medicine and Experimental Therapeutics Branch.

14. None

Honors, Awards, and Publications Sheet

15. NHI-96
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Sidney Udenfriend and James Wyngaarden, Precursors of Epinephrine and Norepinephrine in vivo, Biochem. and Biophysica Acta (in press).

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Chemical Pharmacology — LABORATORY OR BRANCH
3.
4. Goldwater Memorial Hospital, New York, New York — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-97 — SERIAL NO.

6. Biosynthesis and Biotransformation of Ascorbic Acid — PROJECT TITLE

7. John J. Burns — PRINCIPAL INVESTIGATOR(S)

8. Pincus Peyser, Carole Evans, and Julian Kanfer — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To investigate the pathways of biosynthesis and metabolism of ascorbic acid and the factors involved in its physiologic disposition.

Methods Employed: In vivo administration of radioactive precursors. Administration of drugs which increase ascorbic acid formation.

Major Findings: Definitive information has been obtained on the conversion of D-glucose to L-ascorbic acid in the rat. Various compounds markedly increase the biosynthesis of ascorbic acid in the rat, i.e., chloretone, barbiturates, aminopyrine, Butazolidin, etc. Our findings that glucuronic acid is converted to ascorbic acid in the rat suggest that the initial effect of these compounds is to stimulate the synthesis of glucuronic acid. Our results suggest a poly functional role of L-ascorbic acid -- one a non-specific one in which the D-form is effective and the other a specific one which requires the L-form. Further investigation has been carried out on an enzyme system in rat kidney which decarboxylates L-ascorbic acid.

Significance to HEART Research: Vitamin C (ascorbic acid) is involved in maintaining the integrity of all tissues and organs including the heart and cardiovascular system.

Proposed course of project: More detailed studies are planned to investigate the enzyme systems involved in the conversion of D-glucuronolactone and L-gulonolactone to L-ascorbic acid in the rat. Comparative studies will also be carried out with guinea pig tissues to pin point the metabolic block which renders this

species incapable of ascorbic acid biosynthesis. Studies will be continued to determine why various unrelated drugs stimulate the synthesis of ascorbic and glucuronic acids. L-gulonolactone will be administered to patients with essential pentosuria to see whether it effects L-xylulose excretion. In vitro experiments will be continued on the metabolism of L-ascorbic acid, employing 3 differently labelled tracers. The fate of dehydro-L-ascorbic acid will be further studied.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-97
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Dr. Peter Dayton, New York University Research Service, and

Dr. E. H. Mosbach, Columbia University Research Service, at

Goldwater Memorial Hospital

14. None

Honors, Awards, and Publications Sheet

15. NHI-97
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

J. J. Burns, Peter G. Dayton, and Shirley Schulenberg. Further Observation on the Metabolism of L-ascorbic Acid in Guinea Pigs, Journal of Biological Chemistry, in press.

J. J. Burns and E. H. Mosbach. Further Observation on the Biosynthesis of L-ascorbic Acid from D-glucose in the Rat. Journal of Biological Chemistry, in press.

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Chemical Pharmacology — LABORATORY OR BRANCH
3.
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-98 — SERIAL NO.
6. Studies on the Blood-Brain Barrier — PROJECT TITLE
7. Bernard B. Brodie and Steven Mayer — PRINCIPAL INVESTIGATOR(S)
8. Roger Maickel — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION

Objectives: The aim of this project is to determine the relationship between chemical structure of substances of pharmacological interest and the rate at which they enter the brain and cerebrospinal fluid from the blood.

Methods Employed: Analytical methods for substances of pharmacological interest meeting the following criteria: High sensitivity and specificity; substances which are not metabolized or localized in tissues or which can be infused for the duration of the experiment at rates which are calculated from their rates of breakdown or localization. This last criterion is necessary to maintain constant plasma levels of the substances studied. Operative procedures are aimed at maintaining the animals under conditions approaching the normal physiologic state.

Major Findings: (1) Substances injected into the lateral ventricles of dogs disappear at a rate related to their lipid solubility. (2) Infusion of several compounds into a common carotid artery of cats indicates that these substances must be present in a threshold concentration in the blood perfusing the brain in order to produce any significant transfer across the blood-brain barrier. (3) The intravenous administration of small amounts of antipyrine derivatives to rabbits demonstrates rapid equilibration between plasma and brain, but the ratio of brain/plasma is only about 0.6. This is true even of animals sacrificed three hours after injection. With larger doses the equilibrium ratio of brain/plasma approaches 1.

Significance to HEART Research: Many drugs used in cardiovascular diseases act through the central nervous system but little is known about the structural requirements for drugs to pass the blood-brain barrier.

NHI-98
SERIAL NO.

Proposed course of project: Series of related compounds such as the sulfonamides and acridines will be studied in order to relate relative rates of entrance into the brain to differences in properties such as pK, lipid solubility, etc. Two observations that have been made in the past with single compounds require further study with series of related substances: the fact that bromide ion must be present in a minimal concentration in order that any of it appear in the central nervous system; the observation that the amount of barbital which appears in the dog brain at a given time after administration and before equilibrium has been reached is independent of the plasma concentration of the drug.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-98
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Honors, Awards, and Publications Sheet

15. NHI-98
SERIAL NO.

16. NONE

17. NONE

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology
LABORATORY OR BRANCH

3.

4.
LOCATION (IF OTHER THAN BETHESDA)

5. NHI-99
SERIAL NO.

6. Model Enzyme Systems in the Study of Drug Metabolism
PROJECT TITLE

7. Bert N. La Du, Jr.
PRINCIPAL INVESTIGATOR(S)

8. James Gillette and James V. Dingell
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Many drugs are oxidized by liver microsomes in the presence of TPNH and oxygen. The mechanism of these oxidations is still unknown but peroxide may be involved. Some of these drugs can be oxidized to the same products by model catalysts and a study of these model systems may help our understanding of the microsomal enzyme systems.

Methods Employed: Drug metabolism is being studied using various model systems and these reactions compared with the liver microsomal systems. The products are measured by specific microchemical methods.

Major Findings: Various alkylamine drugs which are dealkylated by liver microsomes are also dealkylated by hemoglobin or cytochrome c in the presence of H_2O_2. Normally occurring alkylamines, such as sarcosine or choline are not dealkylated by these systems. Further studies are in progress with the other types of drug metabolism using simple model catalysts.

Significance to HEART Research: Most drugs are extensively metabolized in the body and their pharmacologic effectiveness depends upon how effectively these detoxication mechanisms operate. A study of these model systems may help us learn more about the mechanism of the liver detoxication enzyme systems.

Proposed course of project: During the coming year various other catalysts will be tested with drug substrates and the mechanism of these reactions will be determined.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-99
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-99
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Chemical Pharmacology — LABORATORY OR BRANCH
3.
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-100 — SERIAL NO.
6. Microsomal Drug Enzyme - Mechanism of Action — PROJECT TITLE
7. Bernard B. Brodie and Bert N. La Du, Jr. — PRINCIPAL INVESTIGATOR(S)
8. James Gillette — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION

Objectives: To establish how various types of drugs are metabolized in the body and whether specific detoxication mechanisms exist.

Methods Employed: Drug metabolism pathways are studied in vivo and in vitro and enzyme systems catalyzing these reactions are studied in detail. Metabolic products are measured by specific microchemical methods.

Major Findings: Many drugs are metabolized by liver microsomes and require TPNH and oxygen. A study of these requirements has shown that microsomes contain a TPNH oxidase which yields peroxide. The participation of TPNH oxidase in drug metabolism is indicated since compounds which inhibit TPNH oxidase also inhibit drug metabolism. The possibility that the drug enzymes are a series of peroxidases is unlikely since enzymatically generated peroxide cannot replace TPNH.

Significance to HEART Research: Most drugs are extensively metabolized in the body and their pharmacologic effectiveness depends in part on how effectively these detoxication mechanisms operate. Understanding these mechanisms may be helpful in developing new drugs of longer or shorter duration. Since the detoxication mechanisms vary in different animal species, the observed differences in toxicity and response to drugs in different species may now be explained on a biochemical level.

Proposed course of project: During the coming year several problems to be investigated are: (1) How many types of drug metabolism are present in liver microsomes, (2) are these special mechanisms for drugs and foreign compounds, and (3) how do they operate (mechanism)?

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-100
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-100
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

J. R. Gillette, L. N. La Du, and B. B. Brodie. Further Studies on the Enzymatic Oxidation of Drugs by Liver Microsomes. Journal of Pharmacology and Experimental Therapeutics, in press.

B. B. Brodie, J. Axelrod, J. R. Cooper, L. Gaudette, L. N. La Du, C. Mitoma and S. Udenfriend. Detoxication of Drugs and Other Foreign Compounds by Liver Microsomes. Science, Vol. 121, No. 3147, 1955.

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology
LABORATORY

3.

4.

5. NHI-101
SERIAL NO.

6. Drug Enzyme Systems (Reductive Detoxication)
PROJECT TITLE

7. Bernard B. Brodie and James R. Fouts
PRINCIPAL INVESTIGATORS

8. Jerome Kamm
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION

Objectives: Study of Drug Enzyme Systems

Methods: Micro methods of drug analysis

Major Findings: The reductive metabolisms of aromatic nitro and azo compounds have factors in common. Thus the enzymes which will reduce the nitro group of compounds like chloramphenicol or reductively cleave the azo linkage of drugs like prontosil are present primarily in the liver and kidney of several animal species. Within the liver cell, these enzymes are found only in the microsomes and cytoplasm, and mostly in the cytoplasm. Both require reduced TPN and are activated by all flavins and some dyes, such as indigo.

The in vivo activation of the reduction of p-nitrobenzoic acid to p-aminobenzoic acid has been achieved in mice pretreated with large doses of either riboflavin or FMN.

Significance to HEART Research: The fate of any given drug in the body determines how long it will be effective as well as how toxic it will be. The chemically complex drugs now used in all therapeutics are metabolized by a variety of drug enzyme systems. Understanding these reactions enables one to better assess the overall effects of any given drug.

Proposed course of project: (a) Further study of in vivo activation of drug metabolism is now in progress. (b) The metabolism of other drugs e.g.-chlorpromazine to chlorpromazine sulfoxide -- will be investigated.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-101
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH.

(a) A. K. Saz, Nitro Reductase of Bacteria, NMI, NIH

(b) R. K. Kielley, Reduction of Dinitrophenol by Xanthine Oxidase, NCI, NIH

Honors, Awards, and Publications Sheet

15. NHI-101
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology
LABORATORY

3.

4.

5. NHI-102
SERIAL NO.

6. Enzymatic Dealkylation of Drugs
PROJECT TITLE

7. Bernard B. Brodie
PRINCIPAL INVESTIGATOR

8. Leo E. Gaudette
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION

Project: A study of enzyme systems by which a spectrum of alkylamine drugs and normally occurring N-alkyl compounds are dealkylated.

Objective: To investigate the specificity of enzyme systems responsible for the metabolism of a variety of foreign alkylamine compounds and their differentiation from the systems by which normally occurring alkylamines are metabolized.

Methods Employed: Isolation of particulate cell fractions by the method of Schneider and Hogeboom from various animal tissues. The oxidation of various alkylamine compounds was determined by measuring substrate or product by specific chemical methods.

Major Findings: The dealkylating activity of the microsomal enzyme system of mammalian liver appears to be restricted to foreign alkylamines. None of the normally occurring compounds studied underwent dealkylation.

A study of the distribution of this enzyme system in various species of the phylogenetic scale suggested its predominance essentially in mammals. A variation was also observed in the TPNH oxidation levels in liver microsomes of these species. Generally the variation was such that with decreased TPNH oxidation there was a concomitant decrease in dealkylation activity. Species studied in the order of decreasing activities were rabbit, guinea pig, turtle, frog and fish. The cold-blooded animals showed a predominant decrease in the ability to dealkylate foreign alkylamine drugs.

Significance to Heart Research: The evidence to date would suggest the evolution of a detoxication mechanism in the body for foreign compounds that interfere with its normal economy. A better under-

standing in the use of drugs can be obtained in knowing the character and specificity of a system evolved for their detoxication.

Proposed course of project: The distribution of the microsomal enzyme system will be sought in other species of the phylogenetic scale, and other mechanisms of detoxication will be investigated in species failing to demonstrate dealkylation activity. In addition, further consideration will be given to the differences in requirements of some of the systems active on normally occurring alkylamines, and the extent of their activity on foreign compounds.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-102
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-102
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Bernard B. Brodie, Julius Axelrod, Jack R. Cooper, Leo Gaudette, Bert N. La Du, Chozo Mitoma, and Sidney Udenfriend, Detoxication of Drugs and Other Foreign Compounds by Liver Microsomes. Science 121, 603 (1955).

Bert N. La Du, Leo Gaudette, Natalie Trousof, and Bernard B. Brodie. Enzymatic Dealkylation of Aminopyrine (Pyramidon) and Other Alkylamines. Journal of Biological Chemistry 214: 741 (1955).

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology

3.

4. Goldwater Memorial Hospital
New York, New York
LOCATION

5. NHI-103
SERIAL NO.

6. Studies with Intravenous Anesthetics
PROJECT TITLE

7. Bernard B. Brodie and John J. Burns
PRINCIPAL INVESTIGATOR

8. Natalie Trousof and Constance Glascow
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: A study of the physiologic 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 nonbarbiturate anesthetic since it has become clear that barbiturates as a class are slowly metabolized and exert a hypnotic and not a truly anesthetic action.

Methods Employed: Chemical assay of drugs and their metabolites in blood and tissues.

Major Findings: A steroidal intravenous anesthetic, Viadril, has recently been introduced by Pfizer and Company. Studies are now in progress in an attempt to evaluate its potential usefulness as an intravenous anesthetic. The compound is of great interest to us since, being a steroid, it has a completely different structure than any of the drugs now being used for this purpose. Preliminary results show that the drug disappears very rapidly from the plasma of dogs. The drug has been administered to 10 patients in the operating room at Presbyterian Hospital. In doses of 1 to 1.5 gms. the drug has a definite hypnotic effect. In order to obtain adequate surgical anesthesia, however, it was necessary to supplement its use with nitrous oxide. From these limited experiences it is not possible as yet to come to any conclusion about its role in anesthesiology.

Analysis of NIH Program Activities

Project Description Sheet

NHI-103
SERIAL NO.

Significance to HEART Research: The action of anesthetics on the cardiovascular system in some instances is responsible for their toxicity. Better and safer anesthetics are therefore being sought.

Proposed course of project: As soon as the method for Viadril is perfected further studies will be carried out in its physiologic disposition. We are particularly interested in correlating the rate of penetration of the succinate and/or the free steroid with onset of hypnotic action in dogs. Previously we reported that the rapid onset of action of Pentothal in anesthesia is due to the speed with which it gets into the brain. In fact, the only barrier appears to be the rate of cerebral blood flow. Experiments are planned with other anesthetics, i.e., Evipal, Surital and Kemithal to see whether they also owe their rapid onset of action to rapid penetration into the brain.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-103
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Department of Anesthesiology, College of Physicians and Surgeons, Columbia University, and New York University Research Service, Goldwater Memorial Hospital.

14. None

Honors, Awards, and Publications Sheet

15. NHI-103
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

J. J. Burns, Betty L. Berger, Philip A. Lief, Alfred Wollack, E.M. Papper, and Bernard B. Brodie. The Physiological Disposition and Fate of Meperidine (Demerol) in Man and a Method for its Estimation in Plasma. J. of Pharm. and Exp. Therap. Vol. 114, No. 3, 1955.

E. M. Papper, R. G. Peterson, J. J. Burns, E. Bernstein, P. Lief, and Bernard B. Brodie, Physiological Disposition of Certain N-Alkyl Thiobarbiturates. Anesthesiology, Vol. 16, No. 4, 1955.

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology

3.

4.

5. NHI-104
SERIAL NO.

6. General Drug Screening Program
PROJECT TITLE

7. Neil C. Moran
PRINCIPAL INVESTIGATOR

8. Gertrude P. Quinn and James Watts
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: 1. To screen new synthetic and plant compounds for hypotensive and other actions. 2. To evaluate mechanism and duration of action and toxicity of active compounds. 3. To attempt a pharmacological evaluation of mechanisms of vasomotor regulation.

Methods Employed: Standard physiological techniques for recording blood pressure, blood flow, electrocardiograph in animals.

Major Findings: Of 31 substances screened seven showed interesting cardiovascular activity.

(1) Ocotea leucoxylon alkaloid is a potent adrenergic blocking agent.
(2) Alkaloids from lauraceae species showed vasodepressor actions.
(3) Alkaloids from Hamelia species are vasodepressor, in part due to adrenergic blockade.
(4) Tonduzia parvifolia alkaloid is a ganglionic blocking agent.
(5) Tecoma stans alkaloid is a potent vasodepressor agent of unknown mechanism.
(6) Annona cherimolia alkaloid produces vasodepression of undetermined mechanism.
(7) Cassipourea alba alkaloid is a long-acting ganglionic blocking agent.

Significance to Heart Research: The discovery of several hypotensive drugs illustrates progress in the search for new, less toxic, longer acting drugs for the treatment of hypertension.

Proposed course of project: Continued screening of drugs for possible hypotensive action with studies of mechanims of action of such drugs.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-104
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.

Laboratory of Chemistry of Natural Products, NHI, Source of substance for screening, NHI-70, NHI-72, NHI-76.

14. None

Honors, Awards, and Publications Sheet

15. NHI-104
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology
LABORATORY

3.

4.

5. NHI-105
SERIAL NO.

6. Studies on Antiarrhythmic Drugs
PROJECT TITLE

7. Harriet M. Maling
PRINCIPAL INVESTIGATOR

8. Martha A. Williams
OTHER INVESTIGATOR

9. Project Description

Objective: I. Preliminary screening of drugs for antiarrhythmic activity. II. Detailed studies of antiarrhythmic drugs in terms of toxicity, duration and mechanism of antiarrhythmic action, and other pharmacologic actions.

Methods Employed: I. Petroleum ether-epinephrine ventricular and nodal arrhythmias in vagotomized, pentobarbitalized cats.

II. Delayed spontaneous ventricular arrhythmias in unanesthetized dogs following coronary ligation.

Major Findings: The most promising antiarrhythmic drugs studied during the year 1955 are MC-2606 (a drug closely related to procaine amide), mephentermine sulfate (a sympathomimetic amine), chlorpromazine, and pressocaine (a pressor local anesthetic).

Significance to Heart Research: Possible development for clinical use of antiarrhythmic drugs superior to those now available.

Proposed course of project: 1. Continued screening of drugs. 2. Studies on the antiarrhythmic properties of sympathomimetic amines. An analysis of the relation between protective dose against petroleum ether epinephrine arrhythmias in vagotomized cats and the rate of infusion of the sympathomimetic amine. A comparison of the effects produced by different sympathomimetic amines on the spontaneous arrhythmias in dogs following ligation of the anterior descending coronary artery. 3. Further detailed studied on pressocaine, a pressor local anesthetic which exhibits antiarrhythmic action without a fall in blood pressure.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-105
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Clinical screening by Dr. Kayden, Goldwater Memorial Hospital

Dr. Sjoerdsma, NHI supervised several clinical trials with SN 2157.

14. None

Honors, Awards, and Publications Sheet

15. NHI-105
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology
LABORATORY

3.

4. Goldwater Memorial Hospital
New York, New York
LOCATION

5. NHI-106
SERIAL NO.

6. Studies on Anti-Rheumatic Drugs
PROJECT TITLE

7. Bernard B. Brodie and J. J. Burns
PRINCIPAL INVESTIGATORS

8. James Perel and Constance Goascow
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: Screening of non-sterodial, anti-inflammatory agents in man.

Methods Employed: Micro methods for drug analysis, micro isolation techniques for isolation of metabolites, clinical evaluation of the anti-rheumatic effects of various drugs.

Major Findings: Previously we reported that Butazolidin is metabolized in man along two pathways -- one involving the introduction of a hydroxyl group in the para position of one of the benzene rings to form a phenol (Metabolite I), and the other the introduction of a hydroxyl group in the gamma position of the butyl side chain to yield an alcohol (Metabolite II). Both of these metabolites were found to be physiologically active in man. The phenolic derivative has anti-inflammatory and sodium retaining effects comparable to those of Butazolidin. The alcohol metabolite is a potent uricosuric agent. Two compounds were synthesized which had methyl and chloro groups in both para positions of the benzene rings of Butazolidin. Preliminary results show that two other Butazolidin analogues have marked uricosuric effects. The antiinflammatory effect of a new type of agent made available by Hoffmann-La Roche Laboratories was evaluated. This compound had very potent anti-inflammatory effects in the animal tests. Its chemical structure is completely different than Butazolidin.

Significance to HEART Research: A suitable non-steroidal anti-rheumatic agent would be of value not only in the treatment of rheumatoid arthritis, gout, etc., but also in the treatment of rheumatic fever.

Analysis of NIH Program Activities

Project Description Sheet NHI-106
SERIAL NO.

Proposed course of project: Studies will be continued in an attempt to identify further metabolites of Butazolidin. The clinical evaluation of G-25671 in chronic gout will be continued and that of Metabolite I undertaken. Work will continue with our screening of a series of Butazolidin derivatives prepared for us by the Geigy Laboratory. Particular attention will be directed to those compounds which contain substitution in the benzene rings in other than para positions.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-106
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Dr. J. Murray Steele and Dr. Arnold Ritterband of the New York University Research, Goldwater Memorial Hospital, Res. Serv. and Drs. Alexander Gutman and Tsai Fan Yu of Mt. Sinai Hospital, New York, N.Y.

14. None

Honors, Awards, and Publications Sheet

15. NHI-106
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

T. F. Yu, Bruce C. Paton, Theodore Chenkin, J. J. Burns, B. B. Brodie, and Alexander B. Gutman. Effect of G-25671, a Phenylbutazone Analog, on Urate Clearance and Other Discrete Renal Functions in Gouty Subjects. Evaluation as Uricosuric Agent. Journal of Clinical Investigation (in press).

B. B. Brodie, J. J. Burns, T. F. Yu, and Alexander B. Gutman. Isolation, Identification, and Physiologic Effects of Phenylbutazone Metabolites. Proceedings of the Third European Rheumatism Congress, in press.

Effect of Phenylbutazone (Butazolidin) on Experimentally Induced Ocular Inflammation. Norman Yourish, Bruce Paton, B. B. Brodie, and J. J. Burns. A.M.A. Archives of Ophthal. 53: 264, 1955.

J. J. Burns, Rose K. Rose, Sidney Goodwin, Jules Reichenthal, Evan C. Horning, and Bernard B. Brodie. The Metabolic Fate of Phenylbutazone (Butazolidin) in Man. J. Pharm. and Exp. Therap. 113:481, 1955.

J. J. Burns, Betty L. Berger, Philip A. Lief, Alfred Wollack, and E. M. Papper. The Physiological Disposition and Fate of Meperidine (Demerol) in Man and a Method for its Estimation in Plasma. Journal of Pharmacology and Experimental Thera. 114:289-298, No.3., July 1955.

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

NHI-106
SERIAL NO.

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology
LABORATORY

3.

4.

5. NHI-107
SERIAL NO.

6. Cardio-tonic Drug Screening Program
PROJECT TITLE

7. Marion deV. Cotten and Neil C. Moran
PRINCIPAL INVESTIGATOR

8. James S. Watts
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION

Project: Cardio-tonic Drug Screening Program

Objectives: The objective of this project is to find new cardiac stimulant drugs having little or no direct influence upon the diastolic blood pressure or the electrocardiogram.

Methods Employed: The experiments are conducted in anesthetized dogs with intact circulatory and nervous systems. The force of cardiac contraction is measured with a strain gauge arch sutured directly to the heart muscle. Blood pressure and the electrocardiograms are recorded in the usual manner.

Major Findings: Five specially selected plant extracts were examined and all were found to produce an increase in the force of cardiac contraction. Of these, two appeared to produce increases in contractile force without undesirable changes in the blood pressure or the electrocardiogram.

Significance to Heart Research: There is a need for new cardiac stimulant drugs for use in cardiac depressive states. Such new drugs should have relatively powerful effects upon the contractile power of the heart but should lack significant effects upon the diastolic blood pressure or electrocardiogram.

Proposed Course of Project: Further examination of specially selected plant extracts or new synthetic compounds will be made to determine their actions upon the contractile force of the heart, blood pressure and electrocardiogram. Specific and detailed studies will be made of any compound which shows promise as a new cardiac stimulant agent.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-107
Serial NO.

12. Research
BUDGET ACTIVITY

13. None

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Laboratory of Chemistry of Natural Products, NHI-76, NHI-80.

Honors, Awards, and Publications Sheet

15. NHI-107
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology Laboratory

3.

4.

5. NHI-108
SERIAL NO.

6. The Pharmacologic Actions of Chlorpromazine Sulfoxide, a Major Metabolite of Chlorpromazine.
PROJECT TITLE

7. Neil C. Moran
PRINCIPAL INVESTIGATOR

8. William M. Butler, Jr.
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION

Objectives: With the isolation of chlorpromazine sulfoxide as a major metabolite of chlorpromazine a study of certain of its pharmacological actions was undertaken, with particular emphasis on a comparison with chlorpromazine.

Methods Employed: Standard physiologic and pharmacologic techniques were employed for measuring blood pressure, anesthesia time and acute toxicity.

Major Findings: The metabolite of chlorpromazine has been found to have the same actions as chlorpromazine but is less active. Postural hypotension is less marked in the case of the sulfoxide and occurs at doses where there is no adrenergic blockade, suggesting a central depression of this reflex. Sedation and barbiturate potentiation are produced by the sulfoxide at 8 to 10 times the doses required with chlorpromazine. In rodents, however, potentiation was minimal at all doses in spite of a lower lethal dose for the sulfoxide.

Significance to HEART Research: The demonstration of an active metabolite of chlorpromazine with less marked postural hypotensive action offers the possibility of a clinically useful drug of this type with a minimum of cardiovascular side effects.

Proposed course of project: The collection of confirmatory data in terms of barbiturate potentiation in dogs and mice will terminate the project.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-108
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-108
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Norman P. Salzman, Neil C. Moran, and Bernard B. Brodie. Isolation and Pharmacological Properties of Chlorpromazine Sulfoxide, a Major Metabolite of Chlorpromazine. Nature, Dec. 10, 1955.

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute (INSTITUTE) 2. Chemical Pharmacology (LABORATORY)

3. 4. 5. NHI-109 (SERIAL NO.)

6. Observations on the Actions of Andromedotoxin in the Contractile Force of the Heart in situ. (PROJECT TITLE)

7. Marion deV. Cotten and Neil C. Moran (PRINCIPAL INVESTIGATORS) 8. James S. Watts (OTHER INVESTIGATOR)

9. PROJECT DESCRIPTION

Project: The Effects of Andromedotoxin on the Contractile Force of the Heart.

Objectives: The objective of this project is to analyze the direct and indirect effects of andromedotoxin on the contractile force of the heart in dogs with intact circulatory and nervous systems.

Methods Employed: The contractile force of the heart was measured with a strain gauge arch attached directly to the hearts of anesthetized dogs. Blood pressure was measured in the usual way with an electronic device. These animals were subjected to adrenalectomy and to selective interruption of various efferent and afferent autonomic pathways. These latter included interruption of the spinal cord, vagus nerves, carotid sinus nerves, and cardiac sympathetic nerves.

Major Findings: Andromedotoxin produced substantial increases in contractile force and blood pressure in intact dogs. Interruption of the cardiac sympathetic fibers alone or bilateral adrenalectomy alone did not substantially reduce the effects in the intact dogs. Interruption of the spinal cord almost completely abolished these effects. Injection of large doses of andromedotoxin into dogs with interrupted spinal cords produced a slight direct increase in contractile force. These results suggest that the effects of andromedotoxin in intact dogs are largely due to stimulation of sympathetic centers.

Significance to Heart Research: This project was initiated because of previous demonstrations that andromedotoxin produced substantial increases in contractile force in intact dogs, but only minimal increases in the isolated heart. The results of this project show that the contractile force of the heart may be substantially increased by the action of a drug upon sympathetic centers in the brain

as well as by a direct effect of the drug upon the heart.

Proposed Course of Project: This project is completed and no further experiments are planned.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-109
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-109
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology
LABORATORY

3.

4.

5. NHI-110
SERIAL NO.

6. Studies of the Pharmacologic Action of NHI 196, a Hypotensive Alkaloid from the Seeds of Ormosia panamensis, a Central American Tree.
PROJECT TITLE

7. Neil C. Moran
PRINCIPAL INVESTIGATOR

8. Gertrude P. Quinn and William M. Butler, Jr.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The study of the character and mechanism of the actions of NHI-196, an alkaloid from Ormosia panamensis; evaluation of the compound in terms of its hypotensive effectiveness, toxicity, absorption for clinical trial in the treatment of hypertension.

Methods Employed: Conventional physiological techniques for recording blood pressure and flow, neuro-muscular junction transmission, respiration, etc., were employed.

Major Findings: (1) NHI-196 is a potent long-lasting vasodepressor agent. It produces cutaneous flush, hemoconcentration and tachyphylaxis. The site of action is presumably on the vascular smooth muscle, although a component of vasomotor center depression is suggested. (2) Additional actions include myoneural blockade, ganglionic blockade at high doses, intestinal stimulation, bronchoconstriction, and possible potentiation of barbiturate anesthesia. (3) No histological or hematological toxic effects can be demonstrated in dogs and rats with daily treatment for three weeks.

Significance to HEART Research: (1) The possible development of a new effective antihypertensive drug for clinical use indicates progress in the search for more effective, less toxic drugs in this field. (2) The characterization of the mechanism of the hypotensive action may add to the knowledge of blood pressure controlling mechanisms.

Analysis of NIH Program Activities

NHI-110
SERIAL NO.

Project Description Sheet

Proposed course of project: Continued research into the mechanism of the hypotensive action with attempts to evaluate a possible central action.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-110
SERIAL NO.

12. RESEARCH
BUDGET ACTIVITY

13. IDENTIFY AND COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.

Laboratory of Chemistry of Natural Products, NHI, Chemical study of alkaloid and supply of material for pharmacological study, NHI-77.

14. None

Honors, Awards, and Publications Sheet

15. NHI-110
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology
LABORATORY

3.

4.

5. NHI-111
SERIAL NO.

6. Influence of Reflex Sympathetic Nervous Activity Upon the Force of Ventricular Contraction and Heart Rate.
PROJECT TITLE

7. Marion deV. Cotten and Neil C. Moran
PRINCIPAL INVESTIGATORS

8. James S. Watts
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION

Project: Reflex Control of Cardiac Contractile Force.

Objectives: The objective of this project is to study the extent to which reflex sympathetic nervous activity can influence the force of cardiac contraction and heart rate.

Methods Employed: Anesthetized dogs with intact nervous and circulatory systems were employed in which direct measurement of contractile force was made with a strain gauge arch. Blood pressure was measured in the usual manner with an electronic device. Reflex increase in sympathetic activity was produced by temporary occlusion of the common carotid arteries.

Major Findings: Electrical stimulation of the cardiac accelerator nerves produced a marked increase in contractile force and heart rate, but little increase in diastolic blood pressure. Reflex increases in sympathetic activity following occlusion of the common carotid arteries produced a marked increase in blood pressure and heart rate but little increase in contractile force. Bilateral adrenalectomy alone or removal of the cardiac sympathetic nerves alone did not decrease the minimal increase in contractile force produced by carotid occlusion. A slight increase in contractile force and a marked increase in blood pressure was also produced by methoxamine, a vasoconstrictor agent having no direct action on the heart. It appears that reflex increases in sympathetic activity produced by carotid occlusion do not increase cardiac contractile force to any marked extent.

Significance to Heart Research: Little is known of the role of the sympathetic nervous system with regard to the control of the cardiac contractile force. Experiments such as those described above will provide interesting information regarding this important function.

Analysis of NIH Program Activities

Project Description Sheet NHI-111
SERIAL NO.

Proposed Course of Project: Further experiments will be conducted using other methods for increasing reflexly the activity of the sympathetic nervous system to determine what nervous pathways are concerned with the reflex control of the contractile power of the heart muscle.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-111
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-111
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute — INSTITUTE
2. Chemical Pharmacology — LABORATORY OR BRANCH
3.
4. — LOCATION (IF OTHER THAN BETHESDA)
5. NHI-112 — SERIAL NO.

6. Relationship Between Stroke Work and Ventricular Contractile Force — PROJECT TITLE

7. Marion DeV. Cotten and Harriet M. Maling — PRINCIPAL INVESTIGATOR(S)

8. Martha A. Williams and James S. Watts — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objective of this project is to examine the relationships which exist between changes in the stroke work of the heart and changes in the force of the ventricular contractions.

Methods Employed: These experiments are conducted in the denervated heart-lung preparation of the dog. Stroke work is calculated from the arterial pressure, stroke volume and atrial pressure. Ventricular contractile force is measured with a strain gauge arch attached directly to the ventricular muscle. Stroke work and contractile force are increased both by increasing the atrial pressure in graded increments and by norepinephrine.

Major Findings: Ventricular function curves similar to those observed in intact dogs are obtained when atrial pressures are increased progressively from control values up to 250 mm. water. There is a linear or slightly curvi-linear relationship between the stroke work and the force of the ventricular contractions when these latter parameters are increased both by raising the atrial pressure and by an injection of norepinephrine.

Significance to HEART Research: In order to measure the actions of cardiac stimulant or cardiac depressant drugs upon the contractile force of heart muscle, it is desirable to make such measurements directly under conditions of an intact circulatory system. This project is concerned with examining the contractile force of the heart. The strain gauge arch provides a simple method for measuring changes in contractile force and the demonstration of the direct relationship between such measurements and the stroke work shows that the strain gauge arch may be substituted reliably for more complicated and indirect methods.

NHI-112
SERIAL NO.

Proposed course of project: Additional experiments will be conducted to confirm the findings described above. The same methods and procedures used previously will be employed in these experiments.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-112
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-112
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology
LABORATORY

3.

4.

5. NHI-113
SERIAL NO.

6. Absorption of Drugs by the Intestine
PROJECT TITLE

7. Bernard B. Brodie
PRINCIPAL INVESTIGATOR

8. Lewis S. Schanker
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION

Project: A quantitative study of drug absorption using the rat small intestine in situ.

Objectives: To determine which of the chemical and physical properties of drugs govern their rates of absorption.

Methods Employed: A series of drugs has been studied using the intact small intestine of the anesthetized rat. The intestine is cannulated at the pylorus and at the ileocecal junction and is then perfused at a constant rate with an isotonic saline solution (pH 7.2) containing the drug at a concentration of 1.0 mM. The perfusion solution also contains radioinulin as a volume change indicator. The extent of drug absorption is determined by the concentration difference between the solution entering the duodenum and leaving the ileum.

Major Findings: The rates of absorption of organic bases appear to be related to the ionization constants, weaker bases being well absorbed and stronger bases poorly absorbed. Such a relationship is not apparent for the absorption of organic acids.

Significance to HEART Research: A knowledge of the factors governing drug absorption should be of value in the selection of therapeutic agents for treatment of cardiovascular disease.

Proposed course of project: To extend the series of drugs, especially the organic acids. To investigate the contributions of plasma binding, lipid solubility and the ionization constant to the rate of drug absorption.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-113
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Dr. C. Adrian M. Hogben, Laboratory of Kidney and Electrolyte Metabolism, NHI-22

14. None

Honors, Awards, and Publications Sheet

15. NHI-113
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology
LABORATORY

3.

4.

5. NHI-114
SERIAL NO.

6. Epinephrine and Norepinephrine Arrhythmias in Dogs
PROJECT TITLE

7. Harriet M. Maling
PRINCIPAL INVESTIGATOR

8. Martha A. Williams
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION

Objective: A study of the electrocardiographic responses of unanesthetized dogs to intravenous injections of epinephrine and norepinephrine during the first fifteen days following coronary ligation. Elucidation of the cause or causes of the exaggerated arrhythmias induced by epinephrine and norepinephrine in the unanesthetized dog after coronary ligation.

Methods employed: Intravenous injections and simultaneous recordings of blood pressure and ECG in unanesthetized dogs, both before and after coronary ligation.

Major Findings: It was found that epinephrine and norepinephrine induce arrhythmias in dogs during the first 15 days following coronary ligation. Following the operation there was a two- to fourfold increase in sensitivity to each drug. Norepinephrine produced longer lasting arrhythmias and usually a higher maximum rate of ectopic rhythms than the corresponding equimolar dose of epinephrine. These sensitized ectopic responses to epinephrine and norepinephrine were almost completely abolished by atropine, suggesting either exaggerated reflex vagal activity following coronary ligation, or masking of ectopic activity by the high sinus rate resulting from the atropine.

Significance to HEART Research: Better understanding of the changes in the physiology of the heart resulting from coronary ligation.

Proposed course of project: A. The ECG responses to epinephrine and norepinephrine will be compared with the ECG responses to other sympathomimetic drugs, both before and after coronary ligation, to determine how specific the sensitization is.

B. The ECG responses to epinephrine and norepinephrine will also be compared with the ECG responses to the parasympathomimetic drug methacholine.

C. The effect of atropine upon the spontaneous delayed ectopic activity following coronary ligation will be determined.

D. The theory that a high sinus rate can mask ectopic activity will be tested.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-114
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-114
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology
LABORATORY

3.

4.

5. NHI-115
SERIAL NO.

6. Respiratory Physiology During Anesthesia and Hypothermia
PROJECT TITLE

7. J. W. Severinghaus
PRINCIPAL INVESTIGATOR

8. M. Stupfel and A. Freeman Bradley
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To study the distribution of pulmonary blood flow and ventilation as altered by anesthesia, hypothermia and associated technics.

Methods Employed: By measuring O_2 and CO_2 tensions in arterial blood, and expired air and mixed expired air, and by recording the instantaneous gas tensions during expiration, predictions of the distribution in the lung can be made.

Major findings: Pulmonary blood flow is made uneven by large tidal volumes or by small lung volumes, and is more even at large lung volumes and small tidal volumes. Distribution is improved by hexamethonium. No change is produced in distribution by artificial respiration, chloralose anesthesia, succinyl choline, body position or pattern of breathing. The amount of lung involved in embolic occlusion can be measured. Patients with rheumatic heart disease appear to have a moderate unevenness of distribution of pulmonary blood flow.

Significance to Heart Research: Hypothermia is now used during cardiac surgery in some patients. One of the major unsettled problems at the present is how to maintain the patient in a state that will avoid 1) ventricular fibrillation 2) post operative pulmonary edema. The blood gas tensions and type of ventilation are of importance in this respect.

The preoperative evaluation of patients from the standpoint of lung function needs a method for measuring distribution of blood flow in the lung.

Analysis of NIH Program Activities

Project Description Sheet

NHI-115
SERIAL NO.

Proposed course of project: Measurements are being made on patients and normal adults in an effort to discover what disease entities are associated with pulmonary blood flow distribution abnormalities. It is planned to make these measurements during thoracic surgery.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-115
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-115
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR

Respiratory dead space increase following atropine in man, and atropine, vagal or ganglionic blockade and hypothermia in dogs. J. W. Severinghaus and M. Stupfel J. Appl. Physiol. 8, 81 1955.

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology
LABORATORY

3.

4.

5. NHI-116
SERIAL NO.

6. Studies on Blood pH, pK' of Carbonic Acid and pCO_2 vs. Temperature
PROJECT TITLE

7. J. W. Severinghaus
PRINCIPAL INVESTIGATOR

8. Maurice Stupfel and A. Freeman Bradley
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To improve the accuracy of measurement of blood pH and pCO_2. To study the source of error, and particularly the effect of temperature.

Methods employed: Blood of known pH or known pCO_2 is prepared by equilibration and measurement of CO_2 content.

Major findings: pH meter temperature control has been found extremely important, and an oil bath in an oven is now used. Blood has the effect of lowering the observed pH in a glass electrode 0.01 below its true (plasma) pH. The pK' of carbonic acid is much less variable than was formerly supposed, if measured at exactly 37.5° and pH 7.4. It rises as temperature or pH falls, and these slopes have been determined. Blood pCO_2 has been measured in 20 individuals' bloods with a demonstrated accuracy of 0.3 mm Hg, or 1%.

Significance to heart research: All discovered errors were in the same direction, and occurred with each measurement, so that former measurements were usually incorrect by 5-15%, leading to some misinterpretations of physiologic mechanisms.

Proposed course of project: Completed.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-116
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-116
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology
LABORATORY

3.

4.

5. NHI-117
SERIAL NO.

6. Biogenesis of Aromatic Compounds
PROJECT TITLE

7. Chozo Mitoma
PRINCIPAL INVESTIGATOR

8. Herbert Posner
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION

Objectives: The formation and hydroxylation of aromatic and cyclic compounds are important biochemical processes. These reactions will be investigated, especially the formation of histidine, hydroxyproline, tyrosine, and 5-hydroxytryptophan.

Methods Employed: Standard enzymatic methods.

Major Findings: The enzyme system which catalyzes the conversion of cyclohexane-carboxylic acid to benzoic acid was found in the combined fractions of mitochondria and high speed (100,000 x g) supernatant of rabbit and guinea pig livers. Mitochondria alone did not show any activity. Carbon 2 of guanine is a more direct precursor of histidine than is formate. The transfer of the formamido- or the ureido group of guanine to histidine is a possibility.

Significance to HEART Research: Amines derived from aromatic and cyclic amino acids are humoral agents acting on the cardiovascular system.

Proposed course of project: Further studies will continue on the liver aromatizing system. Studies will be started on the formation of hydroxyproline, in intact animals, tissue slices and in vitro. Substrates, activators and inhibitors of this formation will be investigated.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-117
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Esmond E. Snell, Department of Chemistry, University of Texas

14. None

Honors, Awards, and Publications Sheet

15. NHI-117
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:
Chozo Mitoma and Esmond E. Snell, The role of purine bases as histidine precursors in L. casei. Proc. Nat. Acad. Sci. 41, 891 (1955).

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology
LABORATORY

3.

4.

5. NHI-118
SERIAL NO.

6. Tyrosine Oxidation
PROJECT TITLE

7. Bert N. La Du, Jr.
PRINCIPAL INVESTIGATOR

8. Vincent Zannoni
OTHER INVESTIGATOR

9. PROJECT DESCRIPTION

Objectives: In the oxidation of tyrosine in liver vitamin C is required to hydroxylate one of the intermediary compounds, p-hydroxyphenylpyruvic acid. The role of vitamin C and the mechanism by which the hydroxyl group is introduced into the aromatic ring have not been established.

Methods Employed: The enzyme system catalyzing this hydroxylation reaction has been obtained in a soluble state from dog and rat liver and has been purified by salt and organic solvent fractionation. The reaction has been followed by chemical, manometric and spectrophotometric techniques.

Major Findings: The hydroxylation of p-hydroxyphenylpyruvic acid requires two protein fractions and ascorbic acid. The exact role of each protein fraction is not known, but one has been identified as catalase. L-ascorbic acid can be replaced by several other compounds having about the same redox potential, such as D-ascorbic acid, hydroquinone and 2,6-dichlorophenolindophenol dye. These compounds are all required in their reduced form, presumably to reduce a component in one of the enzymes.

Significance to HEART Research: Hydroxylation is a reaction of general importance; the biosynthesis of tyrosine, "dopa," adrenaline, and thyroxine, for example, requires this type of reaction. The hydroxylation of p-hydroxyphenylpyruvic acid to homogentisic acid is a convenient system to use to study biochemical hydroxylation. The fact that vitamin C is required in this reaction is of further interest since a study can also be made of how this vitamin functions in a specific biochemical process.

Proposed course of project: Continued studies will require further purification of the enzyme systems involved and localizing the position of ascorbic acid in the reaction.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-118
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-118
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

B. N. La Du and V. G. Zannoni. The Tyrosine Oxidation System of Liver. II. Oxidation of p-Hydroxyphenylpyruvic Acid to Homogentisic Acid. Journal of Biological Chemistry, 217: 777 (1955).

B. N. La Du and V. G. Zannoni. The Tyrosine Oxidation of Liver. III. Further Studies on the Oxidation of p-Hydroxyphenylpyruvic Acid. Journal of Biological Chemistry, in press.

B. N. La Du and V. G. Zannoni. A Requirement for Catalase in Tyrosine Metabolism: the Oxidation of p-Hydroxyphenylpyruvic Acid to Homogentisic Acid, Nature, in press.

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Chemical Pharmacology
LABORATORY

3.

4.

5. NHI-119
SERIAL NO.

6. Applications of Spectrophotofluorimetric Assay
PROJECT TITLE

7. Bernard B. Brodie and Sidney Udenfriend
PRINCIPAL INVESTIGATORS

8. Daniel Duggan, Robert Bowman and Bruno Vasta
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To study and apply spectrophotofluorometry as an analytical tool.

Methods Employed: ------

Major Findings: In addition to a laboratory instrument, commercial spectrophotofluorometers are now available. A large number of biologically and pharmacologically important compounds have been found to fluoresce. These include vitamin E, vitamin K, pyridoxine, sex hormones, A.T.P., adrenaline, tryptophan, etc. Methods for assay of some of these compounds in tissues have been developed based on their fluorescence.

Significance to HEART Research: Micro techniques for chemical assay are needed to solve pharmacological and biochemical problems in cardiovascular studies.

Proposed course of project: Commercial models of these instruments will be made available to this group for further study and for applications to analytical problems.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-119
SERIAL NO.

12. Research
BUDGET ACTIVITY

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Dr. Daniel Duggan, Visiting Scientist, American Instrument Co.
Dr. Robert Bowman, Laboratory of Technical Development NHI-120
Mr. Bruno Vasta, Fellow, Farrand Optical Company

14. None

Honors, Awards, and Publications Sheet

15. NHI-119
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

Robert L. Bowman, Patricia A. Caulfield, and Sidney Udenfriend. Spectrophotofluorometric Assay in the Visible and Ultraviolet. Science, 122: 32-33, No. 3157, July 1, 1955.

17. None

Analysis of NIH Program activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY OR BRANCH

5. NHI-120
SERIAL NO.

6. Development of Spectrophotofluorometry and Its Application to Biological Measurements
PROJECT TITLE

7. Robert L. Bowman
PRINCIPAL INVESTIGATOR

8. Dr. Sidney Udenfriend (Laboratory of Chemical Pharmacology)
Dr. D. Duggan - Guest Worker
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: (a) To make a practical spectrophotofluorometer capable of performing, as well or better than our experimental instrument available to other laboratories. (b) To continue to participate in exploring the biochemical-pharmacological applications of fluorescence determinations. (c) To continue to explore methods of eliciting and measuring fluorescence.

Methods Employed: (a) The spectrophotofluorometer assembled in this laboratory, being an experimental model was not a completed commercial design, so it was felt that a manufacturer of instruments could be interested in constructing a more practical form of this device. This would save this laboratory the expense and time of perfecting a practical model, and at the same time make many instruments available. The only company that effectively accepted our invitation was American Instrument Co., who, at present have completed their design. They are presenting the instrument to the Heart Institute for evaluation, and at the same time are entering into a production run of a number of these instruments. Our publication of the method and the publication of the method worked out on this instrument by the Laboratory of Chemical Pharmacology have already created a demand for a number of these instruments in many laboratories throughout the world.

(b) In collaboration with the Laboratory of Chemical Pharmacology, methods of application to fluorometry and biochemical - pharmacological problems are being investigated. Materials of interest are being surveyed for heretofore unknown fluorescence in the ultraviolet.

Methods Employed: continued. (c) The application of very high intensity sources, such as the high pressure mercury lamp and the various flash lamps used in photographic methods, were explored as a means of increasing the ultimate sensitivity of fluorometric methods. The modification of fluorescence emission by change of physical state to convert fluorescence to phosphorescence and the analysis of time course of phosphorescent curves were examined. Intensification of fluorescent yield by electric fields was also examined.

Major Findings: (a) The demand for practical instruments for spectrophotofluorometry has indicated the success of this instrumentation project.

(b) Its possible application to the determination of more than 60 compounds of specific biochemical interest with sensitivity sufficient for measurement of less than a microgram per milliliter indicate the value of the technique.

(c) Methods of modification of fluorescence yield by increased light intensity or physical modification of the state of the sample have not so far indicated that any departure from current practice is desirable.

Significance to HEART Research: Spectrophotofluorometric determination of qualitative characteristics of metabolites and chemical agents of interest to heart research facilitates the study of their disposition in the body. The method also facilitates the quantitative determination of these materials in body fluids.

The application of the method to the tranqualizing drugs and the problems of serotonin activity has been especially related to current problems in basic and applied heart research.

Proposed course of project: This project is essentially complete but as new ideas and the need for adaptations for special problems arise, I expect to participate in further developments.

1 c

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-120
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

NHI-119

14. NONE

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-120
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

"Spectrophotofluorometric Assay in the Visible and Ultraviolet" Robert L. Bowman, Patricia A. Caulfield, Sidney Udenfriend, Science, July 1, 1955, Vol. 122, No. 3157, pages 32-33.

17. NONE

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY OR BRANCH

5. NHI-121
SERIAL NO.

6. Development of Methods for Determining Freezing Point Depression
PROJECT TITLE

7. Robert L. Bowman
PRINCIPAL INVESTIGATOR

8. None

9. PROJECT DESCRIPTION

Objectives: To develop and improve methods for determining osmotic activity of biological fluids.

Since the macro method has enjoyed considerable success, we have tested methods for determining freezing point depressions of micro samples to cooperate with the micro puncture renal physiology program.

Methods Employed: Absolute temperature measurements at the freezing point are unnecessary if standard solutions can be proportionally related to a specific time in a temperature cycle that can be reproduced. On this basis a number of methods of cycling the temperature of a set of unknown standards have been tested, but found unsatisfactory for various reasons. A new method of electrical refrigeration is currently under consideration, but efforts to prepare special alloys and construct the cooling device have failed. A search will be made for commercial sources of the special alloys for testing the technique.

Major Findings: None of the methods tested has proven satisfactory for routine application in its present state.

Significance to HEART Research: In the tissue phase of the dynamics of circulation osmotic activity is an index of the load on the secretory, absorbtive or excretory mechanism.

Proposed Course of Project: To continue to elaborate methods and apparatus for micro freezing point determination.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-121
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-121
SERIAL NO.

16. NONE

17. NONE

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY OR BRANCH

5. NHI-122
SERIAL NO.

6. Development of an Ultramicroanalytic Method for Sodium and Potassium Determination in Micropuncture Samples
PROJECT TITLE

7. Robert L. Bowman
PRINCIPAL INVESTIGATOR

8. None

9. PROJECT DESCRIPTION

Objectives: Development of microanalytic methods for determining sodium, potassium and possibly chloride in micropuncture samples.

To develop an accurate dependable method of analysis of samples of 0.0001 and .01 µl containing $50x10^{-12}$ to $150x10^{-10}$ moles of Na and $50x10^{-10}$ to $5x10^{-10}$ moles of K.

Methods Employed: Apparatus and methods previously developed consisting of a microwave resonance cavity to excite an electrodeless discharge in evacuated quartz tubes and optical systems to analyze the emission being utilized to establish blank levels, methods of handling the sample and standard for evaluation of the method.

Major Findings: The microwave resonance cavity used for excitation has been shown to be capable of producing intense emission of line spectra of the alkali metals, as well as many others from extremely minute quantities of these metals.

Significance to HEART Research: This method is to be applied to the micropuncture investigation of the handling of these cations by the kidney and its relation to water and electrolyte balance.

Proposed course of project: The development of the methods for the metals in question will be continued. An exploration of the potentialities of the method in regard to other metals and anions will be made as the work progresses.

Proposed course of project: (continued)

Methods utilizing the electrodless discharge have been published for the halides which suggest that they may also be determined in the same way. Ca and Mg are also to be explored.

It is planned to pursue this project more actively this year as the micropuncture program with which it will be associated is now under way.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-122
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Analysis of NIH Program Activities

Honors, Awards and Publications Sheet

15. NHI-122
SERIAL NO.

16. NONE

17. NONE

4 a

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY OR BRANCH

5. NHI - 123
SERIAL NO.

6. Development of Prosthetic, Therapeutic or Experimental Devices
PROJECT TITLE

7. Robert L. Bowman
PRINCIPAL INVESTIGATOR

8. Hillary Trantham, John Boretos
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To provide consultation and experimental model facilities to investigators requiring special devices.

Methods employed: In cooperation with the Cardiovascular Hemodynamics Laboratory, we have assisted in design and development of (1) a number of blood vessel coaptation clamps for rapidly anastomosing the aorta without suturing, (2) silastic valves for insertion in the aorta to functionally replace the aortic valve with the expectation that a properly designed valve could be placed in the normal position, replacing an insufficient aortic valve. Dr. I. A. K. McMillan performed the surgery and tested the valves with his valve proving machine. Valves of tricuspid design made up in silastic and arranged mechanically so that only a single suture line was required, were put in dog aortas for testing compatibility of the material, thrombus forming problems, and functional valve action. Relatively successful valve action was obtained in spite of poor mechanical advantage to the leaflets due to the single line of attachment. Pressures were maintained and the material appeared compatible, thrombus problems were still present, but the problems appeared soluble with further development.

The quality of the work and progress attained as evaluated by Dr. McMillan, after a tour of surgical prosthetic programs in United States and Canada, indicated that we had made more, or as much progress, as any he encountered. The work, however, is too incomplete for publication. As Dr. McMillan has returned to England, our plans are to continue to maintain contact with the problem by mail.

4 b

Methods Employed: (continued) In cooperation with the Laboratory of kidney and Electrolyte Metabolism, a successful, inflatable cuff renal artery clamp has been developed. In the same laboratory the program on micropuncture techniques (Dr. T. J. Kennedy and Dr. R. Akers) is receiving our support in the form of instrumentation advice and development of special devices. An electrometric chloride method under development by Dr. Cotlove is receiving support in the form of consultation and experimental model construction. It appears to offer improvement in accuracy and speed, as well as reduction in demands on the operation of the method.

In cooperation with the surgical clinic, a bubble column - artificial lung oxygenator was constructed and tested for performance. It was apparent that extravagent claims have been made for this apparatus, and that the answer to the mechanical oxygenator is not the bubble column.

A new form of lung and pump combination has been planned, and preliminary tests on methods of increasing the permeability of Visking tubing indicates that our design should work, but the minimum size of the diffusion membrane will probably be determined only by performance testing.

Significance to HEART Research: Application of the skills and methods of this laboratory have facilitated the progress of numerous programs, and the significance to heart research is apparent.

Proposed course of project: 1. Continued experimentation on new materials and methods of fabrication to maintain familiarity with them. 2. Continue to cooperate in the application of new material and methods to the cooperative projects, and respond to their ability to utilize our products.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-123
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-123
SERIAL NO.

16. NONE

17. NONE

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY OR BRANCH

5. NHI-124
SERIAL NO.

6. Magnetic Pressure Generator
PROJECT TITLE

7. Frank W. Noble
PRINCIPAL INVESTIGATOR

8. Donald L. Fry, Alexander Mallows, Robert E. Gorman
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Development of a hydraulic pressure generator for testing the dynamic performance of pressure gauges.

Objectives: Information available from manufacturers of blood pressure manometers is incomplete. We wish to study the dynamic performance of several gauges when used with a variety of different needle and catheter systems.

Methods Employed: A magnetically actuated pressure generator has been designed and perfected. This device produces sinusoidal pressure waves continuously adjustable in frequency from zero to over 2000 cycles per second. It will also produce any arbitrary wave form having a spectrum extending from zero to 300 cycles per second.

Major Findings: The major findings of this project are contained in a forthcoming paper concerning the dynamic properties of commercial gauge - catheter systems, authored by Dr. D. L. Fry.

Significance to HEART Research: Rather large errors are inherent in the use of conventional gauge - catheter systems. A study of the type and magnitude of these errors should be useful to the practicing clinician who uses these instruments. The results also provide a basis for the design of better instruments for measuring intra-cardiac pressure.

Proposed Course of Project: This project is complete except for publication. A draft is currently being prepared.

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Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-124
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-124
SERIAL NO.

16. NONE

17. NONE

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY OR BRANCH

5. NHI-125
SERIAL NO.

6. Function Generator and Fourier Analyzer
PROJECT TITLE

7. Frank W. Noble
PRINCIPAL INVESTIGATOR

8. Donald L. Fry, Bert R. Boone, Robert E. Gorman
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: To provide means for easily and quickly obtaining the Fourier Analysis of any biological waveform, and for checking this analysis by synthesis.

Objectives: The results of a Fourier Analysis enable one to specify the performance characteristics required of an instrument which is to be used to record a particular biological waveform.

Methods Employed: Electronic apparatus has been designed and built which will convert a biological curve into an electric wave of suitable frequency for connection into a commercial wave analyzer. The measurement of phase is accomplished by generation of a harmonic series of sine and cosine waves of the proper combination such that when their sum is subtracted from a biological wave a null for all frequencies is produced. The harmonic series is then interpreted to yield the first six terms in the Fourier Series for the biological wave.

Major Findings: A six term Fourier Analysis can be obtained in a time of minutes instead of the days required by the conventional schedule method. The analysis can be checked by the reconstruction of the curve from the analysis and comparing it with the original

Significance to HEART Research: Fourier analysis of biological wave forms allows the specification of the dynamic properties of a recording device. Since the analysis is unique, it provides a sensitive method of catalogueing wave forms. Information concerning the wave propagation properties of arteries may be available from such analyses.

Proposed course of project: This project is complete.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-125
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-125
SERIAL NO.

16. NONE

17. NONE

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY OR BRANCH

5. NHI-126
SERIAL NO.

6. X-ray Image Amplification and Cine-radiography
PROJECT TITLE

7. Frank W. Noble
PRINCIPAL INVESTIGATOR

8. Donald L. Fry, Harold T. Dodge, Bert R. Boone, Robert E. Gorman
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: To provide facilities for making high-speed motion pictures of the fluoroscopic image of man and experimental animals.

Objectives: The objective of this project is to investigate the potentialities and shortcomings of the Philips Image Amplifier when used with the Maurer 16 mm motion picture camera.

Methods Employed: Tests have been made to determine the response speed of the Image Amplifier, since this limits the maximum motion picture frame rate. The resolution of the image tube, the camera lens, and the film are currently being investigated to determine the resolution limit and the component which imposes this limit.

Major Findings: It is possible to make motion pictures of the ventricular border in man at the rate of 80 pictures per second. However, the response speed of the image amplifier limits the useful frame rate to 40 pictures per second, since higher rates simply result in multiple printing of the same pictures. The grain size of the Tri-x film is excessive, and the contrast and resolution are somewhat poorer than expected.

Significance to HEART Research: It is believed that cine-radiographs contain the maximum amount of information which can be obtained from the use of x-ray equipment. The great advantage of the image amplifier technique is that the radiation dosage to both the patient and the operator can be greatly reduced from that required for ordinary cine-radiography.

Proposed course of project: A more satisfactory mechanical mount for the image amplifier and camera will be designed and built. A survey of available motion picture film will be made to determine the most satisfactory types. Resolution tests will be continued until we are satisfied that the ultimate performance for the available equipment has been obtained.

Analysis of NIH Program activities

Budget Data Sheet

10. NHI-126
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI- 126
SERIAL NO.

16. NONE

17. NONE

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY OR BRANCH

5. NHI-127
SERIAL NO.

6. A Catheter Tip Pressure Gauge
PROJECT TITLE

7. Frank W. Noble
PRINCIPAL INVESTIGATOR

8. Bert R. Boone, Robert E. Gorman
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Development of a pressure gauge which can be attached to the tip of a cardiac catheter.

Objectives: The objective of this project is to provide means for obtaining accurate intra-cardiac pressures.

Methods Employed: The fluid column of a conventional gauge-catheter system adversely affects the performance of the system. The speed of response is greatly reduced, and the whip of the catheter in the beating heart causes the registration of spurious pressures. A gauge attached to the tip of the catheter will eliminate both sources of error. We have devised a tip gauge based on the principle of a sonic valve. Audible sound is introduced into one lumen of a double lumen catheter. At the tip the sound encounters a valve actuated by the intra-cardiac pressure, and is thereby modulated in accordance with the pressure, the modulated sound is carried back via the second lumen to a recording device, which traces out the pressure on a strip chart.

Major Findings: Tests of the new gauge show it to be uniform in response from zero to 100 cycles per second. The output voltage is very nearly linearly related to pressure over the range from zero to 250 mm Hg. The gauge is practically insensitive to acceleration in any direction, and should therefore produce no errors due to whip.

Significance to HEART Research: A stable, accurate, easily operated intracardiac pressure gauge would overcome the large errors inherent in the use of conventional catheter systems.

Proposed course of project: The gauge will be developed further, both in the electronic instrumentation and mechanical phases. The electronic instrumentation is almost complete, but minor problems remain such as reduction in noise and improvement in stability. The mechanical problems of the valve and catheter system require a considerable amount of development.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-127
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Honors, Awards, and Publications Sheet

15. NHI-127
SERIAL NO.

16. NONE

17. NONE

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY OF BRANCH

5. NHI-128
SERIAL NO.

6. A Direct-coupled Electrokymograph
PROJECT TITLE

7. Frank W. Noble, Bert R. Boone
PRINCIPAL INVESTIGATORS

8. Harold T. Dodge
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: To provide an Electrokymograph having response down to and including zero frequency.

Objectives: The objective of this project is to produce an instrument which will record the motions of the fluoroscopic shadow of the heart. Commercially available instruments employ amplifiers which do not respond to low frequencies. The effect of this is to produce errors in the recording if the heart beat is at all irregular, and to make calibration of the tracing in terms of absolute position very difficult if not impossible. We propose to eliminate both of these difficulties by designing an instrument which responds to d. c.

Methods Employed: An electrokymograph having uniform response from zero to 30 cycles per second has been designed and built. It is being used by Dr. Dodge to determine its suitability from the operational point of view and to compare its performance with the Cambridge Electrokymograph.

Major Findings: Test results indicate that this instrument should prove to be superior to the commercial Electrokymograph in regard to stability, speed, accuracy, and ease of calibration.

Significance to HEART Research: The Electrokymograph is already established as a useful instrument for studying the characteristic motions of the heart border in health and disease. It is hoped that the d. c. response feature of this new instrument will enhance the usefulness of the Electrokymograph by decreasing errors and allowing for easy calibration.

Proposed course of project: A set of rotating cams will be made to test the accuracy of this new instrument and to reveal the type and magnitude of errors produced by the commercial instrument. The new EKY will be used clinically by Dr. Dodge.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-128
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Honors, Awards, and Publications Sheet

15. NHI-128
SERIAL NO.

16. NONE

17. NONE

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY OR BRANCH

5. NHI-129
SERIAL NO.

6. Development of a Probabilistic Model for Growth
PROJECT TITLE

7. Murray Eden
PRINCIPAL INVESTIGATOR

8. Jean Caha
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To construct a minimal information, stochastic, two-dimensional model of a growing organism and to study the properties of the model.

Methods employed: Combinational analysis and probability theory are the basis for most of the analysis. A high speed digital computer is necessary for certain of the computations.

Major Findings: Methods have been developed to facilitate the study of the model by hand computation. A program has been developed to translate the model into a problem suitable for computation by the high speed computer.

Significance to HEART Research: Theoretical studies of this sort are intended to throw some light on the nature of the process that specifies and limits the patterns of growth and repair.

Proposed course of project: The sample of configurations of 1024 cells will be generated and studied using the Institute for Advanced Study Computer. It is anticipated that a modification of the generating procedure will permit the preparation of configurations that qualitatively duplicate the stressed tissue culture studies of Weiss.

If a suitable organism is found (presumably an alga) attempts will be made to investigate the growth of algal colonies and compare it to the predictions of the model.

10 b Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-129
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NOS. ITEM

Personnel - Department of Mathematics, Princeton University
Facilities - Computer Laboratory, Institute for Advanced Study

14. NONE

Honors, Awards, and Publications Sheet

15. NHI-129
SERIAL NO.

16. NONE

17. NONE

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY OR BRANCH

5. NHI-130
SERIAL NO.

6. Development of a Micro Glass Electrode
PROJECT TITLE

7. Murray Eden
PRINCIPAL INVESTIGATOR

8. None

9. PROJECT DESCRIPTION

Objectives: 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.

Methods Employed: Micro glass electrodes are fabricated from special pH responsive glass using techniques similar to those required for the preparation of micro dissection apparatus. Measurements are made with extremely sensitive amplifiers.

Major Findings: Electrodes may readily be made with tips in the range of 2 to 5 microns wide. Such electrodes have very much higher resistance than ordinary glass electrodes, and in consequence they require very good electrical shielding and special measuring techniques.

Significance to HEART Research: Such electrodes may be used to study the acidity in small capillaries or in kidney glomeruli and ultimately within cells.

Proprosed course of Project: Efforts will be made to simplify the preparative treatment in order to cut down on the number of electrodes broken during the handling. Investigation of possible materials for coating the body of the electrode will be continued. The pH response of these high resistance electrodes will be studied using a vibrating reed electrometer that is on order.

11 b Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-130
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Honors, Awards, and Publications Sheet

15. NHI-130
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

"The pH of Rat Tumors Measured In Vivo", M. Eden, B. Haines and H. Kahler, National Cancer Institute, Bethesda 14, Md. 16, 541 (1955)

17. NONE

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY OR BRANCH

5. NHI-131
SERIAL NO.

6. Development of Equipment for Studies of Adsorption
PROJECT TITLE

7. Murray Eden
PRINCIPAL INVESTIGATOR

8. None

9. PROJECT DESCRIPTION

Objectives: To develop methods for determining the thermodynamic properties associated with the adsorption of water on biological substances.

Methods Employed: Samples of biological materials e. g., protein solutions are placed in a thermostated bath. The vapor pressure of the solution is measured by a pressure transducer. A known amount of the water is removed by pumping and the pressure measurement is repeated until the sample is completely dried.

Major Findings: Equipment has been built to make the precise pressure measurements and the precise volume determinations required by the method.

Significance to HEART Research: All biological materials as they exist in the tissues are surrounded by a more or less tight layer of water molecules. Many of the functions of cells seem to depend on the properties of membrane or cell wall. The membrane structure in turn is influenced by the degree of hydration.

Proposed course of project: It is proposed to study hydration of preparations of red blood cell "ghosts", i. e., the membranes left after the contents of the cell have been removed and to determine how this varies with pH, temperature and the age of the blood cells from which they have been obtained.

12 b

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-131
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Honors, Awards, and Publications Sheet

15. NHI-131
SERIAL NO.

16. NONE

17. NONE

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY OR BRANCH

5. NHI-132
SERIAL NO.

6. To Develop a Capillary Viscometer Capable of Measuring Viscosities at Low Sheer Rate
PROJECT TITLE

7. Murray Eden
PRINCIPAL INVESTIGATOR

8. Jean Caha
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objective: To study the kinetics of degradation of high molecular weight biological substances, such as desoxyribonucleic acid or hyaluronic acid.

Methods Employed: The capillary viscometer is designed in such way that there is no change in hydrostatic pressure during the course of the determination. An external very small constant pressure is applied to force the solution through the capillary.

Major Findings: It was determined that the most suitable source of constant low pressure is a dead weight tester operating through a large ballast tank.

Significance to HEART Research: Since the high molecular weight substances in the body are continually undergoing degradation and synthesis, a knowledge of their physical properties may be and important factor in understanding the mechanism of the repair of tissue damage and the maintenance of body integrity.

Proposed course of project: The model will first be studied to determine the lower limit of sheer rate that can be investigated. Solutions of a sample of DNA that had been characterized by Rowen, Eden and Kahler, will be studied to determine the kinetics and the activation energy of the enzymatic degradation of nucleic acid.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-132
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Honors, Awards, and Publications Sheet

15. NHI-132
SERIAL NO.

16. none

17. none

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY OR BRANCH

5. NHI-133
SERIAL NO.

6. Ultraviolet Microspectrometry
PROJECT TITLE

7. John L. Stephenson
PRINCIPAL INVESTIGATOR

8. None

9. PROJECT DESCRIPTION

Objectives: To develop techniques of ultraviolet microspectrometry for intracellular studies.

Methods Employed: Ultraviolet light from a monochromator is passed through a microscopic system and onto a detector (usually a photomultiplier), thus permitting extention coefficients of very small samples to be determined.

Major Findings: None

Significance to HEART Research: Would facilitate study of intracellular nucleoproteins.

Proposed course of project: Temporiarly discontinued because of lack of personnel.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-133
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-133
SERIAL NO.

16. NONE

17. NONE

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY OR BRANCH

5. NHI-134
SERIAL NO.

6. Drying of Tissues
PROJECT TITLE

7. John L. Stephenson
PRINCIPAL INVESTIGATOR

8. Geraldine Willett, Arnold Jones
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To obtain information on the nature of the solid liquid Interface in protoplasm.

Methods Employed: A drying apparatus has been set up in which small samples of tissue can be dried at temperatures varying from above room temperature to the temperature of liquid nitrogen, while recording their weight and temperature.

Systems for the automatic recording of the weight and temperature have been developed. The temperature recording system utilizes more or less standard techniques. The e.m.f. difference between a thermocouple embedded in the tissue and a reference couple is fed into a galvanometer is replaced with a Leeds and Northrup d. c. amplifier. The output from this goes into a chart recorder. Full range in the recorder can be varied from 1 to 25 degrees centigrade. The system is relatively free from drift which is of the order of 1 or 2% over a 24 hour period.

The weight recording systems utilizes an old Cambridge 12 cm. strip camera. The image from the cross hair on a delicate spring balance inside the vacuum chamber is projected on the moving film after being rotated 90° in order to convert vertical into horizontal motion. The zero of the system is adjusted by moving the telescope which is mounted on a micrometer slide. This also gives periodic absolute measurements of position. At present the system operates at an optical magnification of about 100. The resolution of the photographic recording is about 1 mm. Using a spring with an elongation of 5 cms. per gram, a weight change of 1/5000 gram can be detected. It is possible that with sufficient isolation of the system from vibration the resolution can be increased by another factor of five.

NHI-134
SERIAL NO.

Major Findings: The system has been calibrated using the presumably known sublimation rate of water. This has revealed a discrepancy between accomodation coefficients as measured in our apparatus and those measured previously

Significance to HEART Research: By increasing information on basic organization protoplasm.

Proposed course of project: The source of the above-mentioned discrepancy is being sought. As soon as this is established, the work will be extended to protein gels.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-134
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO. ITEM

Dr. Isidore Gersh, Department of Anatomy, University of Chicago

14. NONE

Honors, Awards and Publications Sheet

15. NHI-134
SERIAL NO.

16. NONE

17. NONE

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY OR BRANCH

5. NHI-135
SERIAL NO.

6. Physics of Ultra-rapid Freezing of Water, Colloidal Solutions and Protoplasm
PROJECT TITLE

7. John L. Stephenson
PRINCIPAL INVESTIGATOR

8. Geraldine Willett and Arnold Jones
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: (1) To investigate the basic physics of the rapid freezing process in water, colloidal solutions 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.

Methods Employed: The rate of cooling of various systems is measured with small embedded thermocouples. The output of these thermocouples is amplified and recorded from a cathode ray oscilloscope. From the theoretical analysis of these curves the properties of various coolants and various constants relating to ice crystal function during cooling can be determined.

Major Findings: The cooling properties of isopentane, liquid propane and liquid nitrogen have been measured. Liquid propane has been found to be the best coolant. The properties of a coolant can be characterized in terms of an approximate rate of heat transfer at the surface. Using this measured rate, it has been possible to construct a reasonably complete analysis of cooling.

Initial measurements have been made of rates of ice crystal growth and function. On the basis of these preliminary values, it has been estimated that cooling velocities of the order of several thousand degrees per second are necessary, if cooling of an aqueous system to the metastable vitreous state supposed to exist below -130°C is to be achieved. These computed rates agree approximately with measured rates obtained by correlating cooling curves with electron microscopic observations, and the whole theory is in good qualitative agreement with microscopic observations.

NHI-135
SERIAL NO.

Significance to HEART Research: Investigation of the fundamental nature of hydration phenomena, and extension of freezing and drying as a method of fixation and preservation of biological material.

Proposed Course of Project: Cooling curves of water and tissues are being obtained. The final size and number of ice crystals of tissue with known cooling curves will be measured. These data will be analyzed using the above-mentioned theory.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-135
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Honors, Awards, and Publications Sheet

15. NHI-135
SERIAL NO.

16. NONE

17. NONE

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY OR BRANCH

5. NHI-136
SERIAL NO.

6. Infra-Red Microspectrometer
PROJECT TITLE

7. John L. Stephenson
PRINCIPAL INVESTIGATOR

8. NONE

9. PROJECT DESCRIPTION

Objectives: To develop an infra-red spectrometer suitable for obtaining absorption spectra of tissues and cells.

Methods Employed: Infra-red radiation from a monochromator is passed through a reflecting microscope and into a suitable detector, thus permitting absorption spectra of microscopic samples to be obtained.

Major Findings: None

Significance to HEART Research: If techniques can be developed, may be useful in study of intracellular metabolism.

Proposed course of project: The instrument is being completed. In addition, a Perkin-Elmer single beam monochromator has been ordered and will be used to study infra red spectra of gels during drying. Eventually it is hoped to correlate those macroscopic studies with microscopic.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-136
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. NONE

14. NONE

Honors, Awards and Publications Sheet

15. NHI-136
SERIAL NO.

16. NONE

17. NONE

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY OR BRANCH

5. NHI-136 (1)
SERIAL NO.

6. Development of a Self-Balancing Potentiometer
PROJECT TITLE

7. John L. Stephenson
PRINCIPAL INVESTIGATOR

8. Frank Noble, Thomas Willmon
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To develop a potentiometer type circuit of high sensitivity and rapid response, for the measurement of rapid cooling curves, occurring in one second or less and having rapid transients in various parts of the curve.

Methods Employed: The signal to be measured is applied to a galvanometer. The motion of the light spot of the galvanometer is amplified with photoelectric following system consisting of single photomultiplier and one power tube. A fraction of this signal is used to counteract the motion of the galvanometer. The entire signal is used to drive an appropriate recording system: recording millimeter, speedomax, oscilloscope.

Major Findings: The following system has been used with two different galvanometers to give two basic types of instrument. Used with a galvanometer of the LandN 2430 series, it gives a system with a frequency response of 0 to 10 c.p.s. and a full scale (recording millimeter) sensitivity of 10 μv to 10 mv. The feed-back loop has a gain of about 100 to 1000. This system has tremendous stability, having been used almost continuously for a six month period in the 5 mv range with a drift of less than 1 per cent during that time. It has been used in 10 μv range with noise level of order of 1/20 μv and drift of fraction of microvolt in 24 hour period. The frequency response is 0 to 10 c.p.s.

The second system uses a fast oscillograph type galvanometer with natural frequency of 100 c.p.s. With the following system, the frequency response is better than 500 c.p.s. with critical damping and lower full scale sensitivity of about 100 μv. With both instruments and all ranges, signal to noise is about 1/200.

Significance to HEART Research: Instrument will be of use in recording variety of bioelectric phenomena in which both initial transient and d. c. after potential are of interest.

Proposed course of project: Project is essentially complete. Will be written up for publication. Possibly at some future time, further development to increase versatility and sensitivity of system will be undertaken.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-136 (1)
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards and Publications Sheet

15. NHI-136 (1)
SERIAL NO.

16. NONE

17. NONE

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Laboratory of Technical Development
LABORATORY

5. NHI-136 (2)
SERIAL NO.

6. Electron Microscopy
PROJECT TITLE

7. John L. Stephenson
PRINCIPAL INVESTIGATOR

8. Geraldine Willett, Murray Eden
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To further the application of electron microscopy to cytological problems.

Methods Employed: Thin sections of biological material fixed by freezing, then dried, are examined in the electron microscope. Various staining procedures to heighten contrast have been tried.

Major Findings: In collaboration with Dr. Isidore Gersh at the University of Chicago, techniques for the electron microscopy of frozen-dried material have been worked out. These techniques have the great advantage of permitting a variety of specific staining procedures not suitable with osmium fixed material.

Significance to HEART Research: These techniques should open up new approaches in the submicroscopic pathology of cardiovascular disease.

Proposed course of project: The first phase of the project is essentially complete. Further physical study of some of the basic problems in the freezing is planned. In addition a preliminary analysis of the possibility of applying emission microscopy to biological material has been undertaken.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-136 (2)
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM)

This is a cooperative study with Dr. Isidore Gersh, Department of Anatomy, University of Chicago.

14. None

Honors, Awards, and Publications Sheet

15. NHI-136 (2)
SERIAL NO.

16. NONE

17. NONE

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of Surgery
LABORATORY OR BRANCH

5. NHI-137C
SERIAL NO.

6. Clinical Application of Hypothermia and Allied Techniques.
PROJECT TITLE

7. Andrew G. Morrow, M. D.
PRINCIPAL INVESTIGATOR(R)

8. Leo R. Radigan, M. D.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To apply clinically experimental information concerning the usefulness of general hypothermia as an adjunct to cardiac surgery.

Methods Employed: Extensive investigations have been made in the Experimental Laboratory in the general field of hypothermia and its hemodynamic and physiologic effects. This information is presently being employed in patients undergoing hypothermia for the open correction of cardiac anomalies.

Patient Material:

Admissions: 30 Average Stay: 30 days

Major Findings: More than 20 patients have been subjected to general hypothermia in 1955. The usefulness of atriocaval infiltration in the prevention of ventricular fibrillation has been demonstrated. In addition, studies of electrocardiographic, electroencephalographic and hemodynamic changes associated with hypothermia have been made. Patients have undergone hypothermia in conjunction with operations for interatrial septal defect, pulmonic stenosis, aortic stenosis and other lesions.

Analysis of NIH Program Activities

Budget Data Sheet

Significance to HEART Research: Hypothermia provides up to 10 minutes of safe intracardiac surgery. This has proved feasible only by analysis and application of the experimental studies previously performed.

Proposed Course of Project: Increased use will be made of general hypothermia for open cardiac surgery until a safe extracorporeal circulation is available.

10. NHI-137C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-137C
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of Surgery
LABORATORY OR BRANCH

5. NHI-138C
SERIAL NO.

6. Clinical Application of Studies Concerning Intracardiac Shunts
PROJECT TITLE

7. Andrew G. Morrow, M. D.
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: By the use of new techniques to determine more precisely the nature and extent of intracardiac lesions resulting in abnormal blood flow.

Methods Employed: In addition to routine right heart catheterization, the patients are studied by means of differential nitrous oxide levels, the use of special catheters with inflatable balloons and techniques of selective angiocardiography.

Patient Material:

Admissions: 70 Average Stay: 30 days

Major Findings: By means of the special techniques described, it has been possible to demonstrate small intracardiac shunts undetectable by the usual methods. In addition, we are able to actually measure the size of interatrial communications and to locate them in relation to the intracardiac anatomic landmarks.

Significance to HEART Research: Open procedures for the correction of intraatrial septal defects are presently available. Such techniques will shortly be available for the treatment of ventricular septal defects. In order to use economically the operating time afforded, precise knowledge must be obtained beforehand as to the size, location and extent of the defect.

Analysis of NIH Program Activities

Budget Data Sheet

Proposed Course of Projects: These studies will be continued on new patients and are being carried out 6 months after operation in those patients previously studied.

10. NHI-138C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14 None

Honors, Awards, and Publications Sheet

15. NHI-138C
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of Surgery
LABORATORY OR BRANCH

5. NHI-139C
SERIAL NO.

6. Aortic Catheterization in Patients with Aortic Valve Disease
PROJECT TITLE

7. Andrew G. Morrow, M. D.
PRINCIPAL INVESTIGATOR(S)

8. Joseph Roshe, M. D.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine the difference between central and peripheral pulse contours in patients with disease of the aortic valve.

Methods Employed: Central aortic pressure is obtained by passing a catheter from the femoral artery into the ascending aorta. Records are then made of pressure in the central aorta and peripheral arteries.

Patient Material:

Admissions: 25 Average Stay: 30 days

Major Findings: The abnormal pulse contour associated with aortic stenosis is much more prominent in the central aorta than in the peripheral arteries. By this technique minimal aortic stenosis may be detected. A change in amplitude and contour between the central aorta and the peripheral arteries seems, at this time, to reflect the severity of aortic insufficiency.

Significance to HEART Research: Precise characterization of aortic pulse contours have not been made in patients. It is believed that the measurements described will aid materially in the selection and evaluation of patients considered for corrective surgery on the aortic valve.

Analysis of NIH Program Activities

Budget Data Sheet

Proposed Course of Project: The current studies will be continued and in addition, it is believed that by selective angiocardiography further information can be gained about aortic valve function.

10. NHI-139C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publication Sheet

15. NHI-139C
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of Surgery
LABORATORY OR BRANCH

5. NHI-140C
SERIAL NO.

6. Determination and Evaluation of Left Atrial Pressure in Congenital and Acquired Heart Disease
PROJECT TITLE

7. Andrew G. Morrow, M. D.
PRINCIPAL INVESTIGATOR(S)

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine the usefulness of left atrial pressure measurements in the evaluation and treatment of heart disease.

Methods Employed: Left atrial pressure is determined in patients by passing through the left bronchus a suitable needle. The resulting pressure curve is recorded and later correlated with the operative or autopsy diagnosis.

Patient Material:

Admissions: 50 Average Stay: 30 days

Major Findings: Determination of left atrial pressure have been carried out in more than 250 patients with congenital and acquired heart disease. Studies indicate thus far that the left atrial pressure is the best indication of degree of mitral insufficiency accompanying mitral stenosis. In addition, this affords a precise physiologic measurement of the degree of heart failure present when there is no valvular disease.

Significance to HEART Research: Rheumatic valvular heart disease constitutes the largest single group of surgically treatable lesions. The method described aids materially in the selection and postoperative assessment of patients subjected to mitral valve surgery.

Analysis of NIH Program Activities

Budget Data Sheet

Proposed Course of Project: It is planned to continue the correlation of these data in most patients presenting congenital and acquired heart disease.

10. NHI-140C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-140C
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of Surgery
LABORATORY OR BRANCH

5. NHI-141C
SERIAL NO.

6. Valvular Gradient in Evaluating the Patient With Aortic Stenosis
PROJECT TITLE

7. Andrew G. Morrow, M. D.
PRINCIPAL INVESTIGATOR(S)

8. Leo E. Radigan, M. D.
OTHER INVESTIGATORS

9. Project Description

Objectives: To determine the systolic gradient across the aortic valve in patients with aortic valvular stenosis and to utilize this knowledge in the selection of patients for operation and in their postoperative follow-up.

Methods Employed: The aortic valve gradient can be determined by passing a catheter from the left atrium into the ventricle while a simultaneous measurement of aortic pressure is being made.

Patient Material:

Admissions: 25 Average Stay: 30 days

Major Findings: In the patients studied it has been found that a gradient of 100 to 250 mm. of mercury exists across the aortic valve when the patient has symptoms. Gradients of 50 to 60 have been recorded in earlier or mild forms of the disease. Following aortic valvulotomy the effectiveness of the procedure can be objectively measured by the change in the gradient. In successful cases this has been reduced to 10 or 15 mm. of mercury.

Significance to HEART Research: Aortic valvulotomy remains a somewhat hazardous operation and is not recommended for patients unless the stenosis is severe. The technique described provides an objective method of selecting those patients most likely to benefit. We are at the same time able to determine if the technique used at operation was affecting any individual patient.

Analysis of NIH Program Activities

Budget Data Sheet

Proposed Course of Project: Continued studies will be made and modification of the present operative technique will be made if indicated.

10. NHI-141C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-141C
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of Surgery
LABORATORY OR BRANCH

5. NHI-142
SERIAL NO.

6. Experimental Pulmonary Insufficiency
PROJECT TITLE

7. Jerome Harold Kay, M. D.
PRINCIPAL INVESTIGATOR(S)

8. J. Alex Haller, M. D. & Robert A. Gaertner, M. D.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine the physiologic effects of experimental pulmonary valvular insufficiency.

Methods Employed: The leaflets of the pulmonary valve are excised during a period of hypothermia with inflow occlusion. After operative recovery physiologic studies are performed.

Major Findings: Cardiac catheterization at 2 to 3 months reveals no abnormalities. The dogs will be studied periodically over a long period.

Significance to HEART Research: There has been much discussion in clinical circles regarding the necessity for preserving function of the pulmonary valve. This is of importance in the surgical treatment of valvular pulmonic stenosis.

Proposed Course of Project: The animals described will be studied over a period of several years in an attempt to obtain requisite information.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-142
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-142
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of Surgery
LABORATORY OR BRANCH

5. NHI-143
SERIAL NO.

6. Digitalis Tolerance in Hypothermic Animals
PROJECT TITLE

7. Thomas L. Lombardo, M. D.
PRINCIPAL INVESTIGATOR(S)

8. Leo R. Madigan, M. D.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine if digitalis tolerance is altered when the animal or patient is rendered hypothermic.

Methods Employed: Digitalis toxicity is induced in a normothermic dog by a rapidly acting digitalis preparation. The same animal is later studied under similar circumstances when hypothermic.

Major Findings: It has been found that digitalis tolerance is markedly affected by the level of blood carbon dioxide. It has not yet been established whether digitalis tolerance is actually changed by hypothermia.

Significance to HEART Research: Hypothermia is being used clinically to a great degree. It will be extremely important to know whether digitalis dosage should be the same for these patients as for others.

Proposed Course of Project: The investigations described are being continued in the hope that the necessary information will be forthcoming.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-143
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-143
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of Surgery
LABORATORY OR BRANCH

5. NHI-144
SERIAL NO.

6. The Experimental Production and Study of Aortic Insufficiency In Dogs
PROJECT TITLE

7. Joseph Roshe, M. D.
PRINCIPAL INVESTIGATOR

8.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To study, in experimental animals, the hemodynamic effects of acute and chronic aortic insufficiency and to assess the value of surgical procedures designed to correct it.

Methods Employed: Aortic insufficiency is induced by excising a portion of one or more aortic leaflets by means of a special punch. After operative recovery, physiologic studies are performed and artificial valves are inserted.

Major Findings: Marked changes in the character and amplitude of the aortic pulse waves have been demonstrated in these animals. It has also been demonstrated that they have a marked susceptibility to bacterial endocarditis.

Significance to HEART Research: Surgical treatment of aortic insufficiency is now commonly practiced yet there has been a dearth of detailed physiologic information about this lesion. The current studies should be of great help in assaying operative results in patients.

Proposed Course of Project: The susceptibility of animals to endocarditis has provided an exceptional opportunity to study this disease in animals. The relationship of chemotherapy and cardiac stress in the treatment is presently under investigation.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-144
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-144
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

The Experimental Production of Graded Aortic Insufficiency. Surg., Gynec. & Obst. 101:303, 1955.

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of Surgery
LABORATORY OR BRANCH

5. NHI-145
SERIAL NO.

6. Experimental Aortic Stenosis
PROJECT TITLE

7. George C. Kaiser, M. D.
PRINCIPAL INVESTIGATOR(S)

8. Jerome Harold Kay, M. D., and Robert A. Gaertner, M. D.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To produce valvular aortic stenosis experimentally and at the same time develop a suitable technique for open direct vision operations on the aortic valve.

Methods Employed: With hypothermia and inflow occlusion the aorta can be opened for periods of 4 to 6 minutes. During this time the commissures of the aortic valve are sutured together.

Major Findings: Valvular aortic stenosis has been produced in 20 dogs. Physiologic studies have not yet been performed. It was believed that air embolism would be a major problem but this has not been true.

Significance to HEART Research: The present surgical treatment of aortic stenosis is often unsuitable. If a satisfactory open operation can be developed many more patients could receive more effective treatment.

Proposed Course of Project: The experiments will be continued and physiologic studies on those animals with aortic stenosis will be performed.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-145
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-145
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of Surgery
LABORATORY OR BRANCH

5. NHI-146
SERIAL NO.

6. Combined Aortic and Mitral Insufficiency - A Hemodynamic and Therapeutic Study
PROJECT TITLE

7. Joseph Boshe, M. D.
PRINCIPAL INVESTIGATOR(S)

8. J. Alex Haller, M. D.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To study the hemodynamic alterations in dogs with combined aortic and mitral insufficiency and to evaluate the artificial aortic valve in treatment of this combined lesion.

Methods Employed: Mitral and aortic insufficiency have been produced in succession in experimental animals. After operative recovery right and left heart catheterizations are performed and then a Hufnagel artificial aortic valve is placed.

Major Findings: The Hufnagel valve in this combined lesion is strikingly effective in lowering left heart filling pressures.

Significance to HEART Research: The clinical coexistence of mitral insufficiency and aortic insufficiency has been a therapeutic problem. Present studies indicate that such patients would definitely benefit from the successful insertion of an artificial aortic valve.

Proposed Course of Project: Continuing study of the combined lesion is presently in progress.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-146
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-146
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of Surgery
LABORATORY OR BRANCH

5. NHI-147
SERIAL NO.

6. Experimental Use of an Extracorporeal Circulation
PROJECT TITLE

7. Jerome Harold Kay, M. D.
PRINCIPAL INVESTIGATOR(S)

8. Robert A. Gaertner, M. D.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To develop a safe and effective method for total cardiac bypass permitting open intracardiac surgery.

Methods Employed: Arterial blood from a reservoir is perfused into the carotid and coronary arteries of a dog during complete inflow occlusion. The animal is hypothermic.

Major Findings: More than 100 animals have been subjected to this experimental operation. 65% have survived right ventriculotomy for 30 minutes.

Significance to HEART Research: A simple and effective method for prolonged intracardiac surgery has not yet been developed. This technique has been applied in five patients without survival.

Proposed Course of Project: The perfusion described has been discontinued and further experiments, using biological and mechanical oxygenators are being initiated.

Analysis of NIH Program Activities

Budget Data Sheet

10. NII-147
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NII-147
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of Surgery
LABORATORY OR BRANCH

5. NHI-148
SERIAL NO.

6. Production of Endocarditis in Dogs With Aortic Insufficiency
PROJECT TITLE

7. Joseph Roshe, M. D.
PRINCIPAL INVESTIGATOR(S)

8. Benjamin Highman, M. D. (NIAMD), and Paul D. Altland, Ph.D.(NIAMD)
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To determine the relationship between cardiac stress and susceptibility to bacterial endocarditis.

Methods Employed: Aortic insufficiency is produced by the excision of one or more aortic leaflets. After operative recovery the animals are given intravenous inoculations with various bacteria.

Major Findings: Animals with aortic insufficiency are extremely susceptible to bacterial endocarditis. The Hufnagel valve was found of no benefit in protecting them.

Significance to HEART Research: Large animals are notoriously immune to bacterial endocarditis. The present preparation affords an excellent opportunity for the study of the therapy and pathology of this disease.

Proposed Course of Project: The relationship between adrenalectomy and susceptibility to endocarditis is presently under investigation.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-148
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-148
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of Surgery
LABORATORY OR BRANCH

5. NHI-149
SERIAL NO.

6. Coronary Perfusion During Hypothermia
PROJECT TITLE

7. Leo R. Radigan, M. D.
PRINCIPAL INVESTIGATOR(S)

8. Thomas A. Lombardo, M. D.
OTHER INVESTIGATORS

9 PROJECT DESCRIPTION

Objectives: To determine the necessary rate of coronary artery perfusion during total bypass of the heart.

Methods Employed: The coronary arteries are supplied with regulated amounts of arterial blood during a period of complete inflow occlusion. Coronary sinus flow and oxygen content are determined.

Major Findings: The optimal rate for coronary perfusion has been determined at approximately 30 cc./min. under these conditions.

Significance to HEART Research: In all systems of open cardiac surgery which require complete inflow occlusion, the coronaries must be supplied with blood. There is evidence to indicate that excessive amounts of coronary perfusion exercise deleterious effects on the myocardium. Accurate information as to the best rate of perfusion is necessary.

Proposed Course of Project: Studies are in progress to determine the extent to which coronary perfusion influences post-operative myocardial failure.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-149
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None.

14. None

Honors, Awards, and Publications Sheet

15. NHI-149
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of Surgery
LABORATORY OR BRANCH

5. NHI-150
SERIAL NO.

6. Experimental Production of Pulmonary Arteriovenous Fistulae
PROJECT TITLE

7. Robert A. Gaertner, M. D.
PRINCIPAL INVESTIGATOR(S)

8. Jerome Harold Kay, M. D.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To produce experimentally, pulmonary arteriovenous fistulae and study the physiologic and pathologic results of this lesion.

Methods Employed: The fistula is produced by an anastomosis between a lobar pulmonary aretery and vein after lobectomy. The animal is subsequently studied by means of cardiac catheterization, dye dilution curves and hematologic methods.

Major Findings: The lesion described results in a decrease in arterial oxygen saturation to a level of 60 to 70%; polycythemia results.

Significance to HEART Research: The hemodynamic results of pulmonary arteriovenous fistulae have been inadequately described. The experimental preparation should benefit excellent opportunity for such measures.

Proposed Course of Project: Detailed hematologic, radiographic and physiologic studies on these animals are currently in progress.

Analysis of NIH Program Activities

Budget Data Sheet

10. NIH-150
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NIH-150
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of Surgery
LABORATORY OR BRANCH

5. NHI-151
SERIAL NO.

6. Experimental Pulmonary Stenosis
PROJECT TITLE

7. Jerome Harold Kay, M. D.
PRINCIPAL INVESTIGATOR(S)

8. George C. Kaiser, M. D. and Robert A. Gaertner, M. D.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To develop a method for producing valvular pulmonary stenosis and to determine the most effective method of treatment.

Methods Employed: Valvular pulmonary stenosis is produced by suturing the pulmonary leaflets under direct vision with the aid of hypothermia. After operative recovery animals are subjected to cardiac catheterization.

Major Findings: It has been found possible to produce a lesion comparable to that seen in the clinical disease. Right ventricular pressures of 80 to 120 are produced within a few months.

Significance to HEART Research: The surgical treatment of pulmonary stenosis is currently the subject of much debate. These animals will be subjected to various forms of pulmonary valvotomy and the effectiveness of various techniques will be assayed.

Proposed Course of Project: See above.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-151
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-151
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of Surgery
LABORATORY OR BRANCH

5. NHI-152
SERIAL NO.

6. Treatment of Cardiac Arrest
PROJECT TITLE

7. Jerome Harold Kay, M. D.
PRINCIPAL INVESTIGATOR(S)

8. George C. Kaiser, M. D. and Robert A. Gaertner, M. D.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To improve methods for treating ventricular fibrillation and cardiac arrest.

Methods Employed: Ventricular fibrillation is induced in dogs by electric shock and defibrillation is then carried out with different voltages and durations of shocks.

Major Findings: Shocks of 230 volts for 1/4 second cause burns in 90% of animals. Lower voltages appear equally effective in defibrillation without burns.

Significance to HEART Research: Ventricular fibrillation is a common occurrence in clinical cardiac surgery. A sound experimental basis for its treatment should prove a great benefit to all those doing cardiac surgery.

Proposed Course of Project: Studies are to be continued, evaluating various ancillary drugs and procedures used with electric shock.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-152
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications Sheet

15. NHI-152
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

5. NHI-155C
SERIAL NO.

6. Ventricular Dynamics in Pulsus Alternans
PROJECT TITLE

7. Harold T. Dodge, M.D.
PRINCIPAL INVESTIGATOR(S)

8. Miss Clara V. King, Mrs. Margaret Austin
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: The objective of this study is to clarify the mechanism of post-extra-systolic pulsus alternans which is frequently one of the early signs of left ventricular failure.

Methods Employed: Observations will include variations of heart rate, directly recorded arterial pressure, and an index of ventricular volume changes as determined by the electrokymogram. The plan is to study post-extrasystolic left ventricular behavior in a group of patients with normal left ventricles as contrasted of a group with abnormal left ventricles.

Major Findings: Observations to date are few and these uncertain because of problems of instrumentation. The instrumentation problem seems to be largely solved as a result of the cooperation of the instrument section which has made a new type electrokymograph available to us.

Patient Material: For this study two groups of patients are required: 1) patients with premature contractions and no left ventricular disease, and 2) patients with premature contractions and left ventricular disease.

Significance to Heart Research: Information concerning this abnormality with further knowledge concerning the hemodynamics of the failing left ventricle.

Proposed Course of Project: More data are to be collected concerning post-extrasystolic left ventricular function patients with normal and abnormal left ventricles.

Patient Material:

Adult Males	2	11 days
Adult females	1	12 days

Analysis of NIH Program Activities

Budget Data

10. NHI-153C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications

15. NHI-153C
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

5. NHI-154C
SERIAL NO.

6. Ventricular Dynamics in Atrial Fibrillation
PROJECT TITLE

7. Harold T. Dodge, M.D.
PRINCIPAL INVESTIGATOR(S)

8. Frederick T. Kirkham, M.D., Miss Clara V. King, Mrs. Margaret Austin
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: This project was undertaken with two objectives: 1) to clarify the mechanism of left ventricular behavior under conditions of the irregular ventricular rhythm that is a consequence of this arrhythmia and 2) to determine whether or not the beat to beat hemodynamic variations as a consequence of this arrhythmia could be utilized as another means of studying left ventricular function under varying degrees of hemodynamic stress.

Methods Employed: Heart rate has been monitored from the electrocardiogram. Duration of the phases of the heart cycle has been determined from heart sounds. An index of left ventricular volume changes has been determined by the electrokymogram. Left ventricular pressures have been determined from records obtained for us by the surgical group either at surgery or by a catheter utilizing the bronchoscopic technique. From these data information concerning the beat to beat changes of stroke volume and stroke work have been calculated.

Patient Material: Patients with atrial fibrillation have been used in this study. A few more observations need be done to complete the study.

		No.	Average Stay Days
Admissions:	Adult males	8	7 days
	Adult females	15	10 days

Major Findings: In atrial fibrillation there are beat to beat changes of both left ventricular end-diastolic and end-systolic volumes. The end-diastolic volume variations are a function of both filling period and the end-systolic volume of the preceding beat. Stroke work is determined by the left ventricular end-diastolic volume.

Analysis of NIH Program Activities

Project Description Sheet

Significance to Heart Research: These observations not only provide an explanation for certain of the hemodynamic phenomena observed in this arrhythmia, but provide the first demonstration in man of the applicability of Starling's Law of the Heart. Furthermore it offers another approach to the study of heart function in man.

Proposed course of project: This project is nearly completed and the data are being prepared for publication.

Analysis of NIH Program Activities

Budget Data

10. NHI-154C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications

15. NHI-154C
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

2. Clinical Endocrinology
SECTION

5. NHI-155C
SERIAL NO.

6. Study of the Relationship of Adrenal Cortical Steroid Structure to Function
PROJECT TITLE

7. Frederic C. Bartter, M. D., and Maurice M. Pechet, M.D.
PRINCIPAL INVESTIGATOR(S)

8. Blair Bowers, Catherine Delea, Margaret Martin, Sarah Lerner
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Study of the relationship of adrenal cortical steroid structure to function.

Objectives: The object of this project is to attempt by comparison of steroids with analogous compounds bearing a single structural alteration to determine the fundamental relationship of steroid structure to physiologic action.

Methods Employed: Metabolic balance studies were conducted in normal volunteer subjects and in patients with Addison's disease. Studies of renal function were performed at intervals throughout the studies. Assays for the acute effects of the steroids on renal sodium and potassium excretion were carried out in dogs.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	3	90
	Adult females	6	120
	Children male	0	0
	Children female	0	0
Outpatient	Number of patients	0	
	Number of visits	0	

Major Findings: It was found that introduction of a double bond between Carbon 1 and Carbon 2 of steroids increased the "carbohydrates" activity, (as assayed from eosinopenic effect and ability to produce negative nitrogen balance) of those steroids possessing an oxygen function on Carbon 11. In steroids with no C-11 oxygen the unsaturation had no effect: in particular it did not increase sodium retention. Under balance conditions a number of the unsaturated 11-oxygenated steroids produced sodium loss as compared with their saturated analogs. It was postulated that this was a result of enhancement of filtration rate, a property of the steroids which

closely paralleled "carbohydrate" activity, without concomitant increase in sodium transport-activity.

Proposed course of project: Newer steroids will be tested as they are made available, largely through pharmaceutical houses. The close cooperation of the pharmaceutical houses in synthesizing steroids designed to elucidate mechanisms has been an indispensable part of this program. Syntheses have not been attempted in this department in view of this cooperation.

Significance to Heart Research: These studies are designed to elucidate the mechanism whereby steroids exert their characteristic actions. This should contribute both understanding of the basic processes involved in inflammatory diseases (e.g., rheumatic fever) and those involved in pathologic salt retention. It is hoped that they may also give rise to steroids effective either directly or through competition with endogenous mechanisms.

Analysis of NIH Program Activities

Budget Data

10. NHI-155C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14 None

Honors, Awards, and Publications

15. NHI-155C
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

5. NHI-156C
SERIAL NO.

6. Studies of the Role of Adrenal Cortical Steroids in the Sodium Retention of the Edematous State.
PROJECT TITLE

7. Frederic C. Bartter, M.D.
PRINCIPAL INVESTIGATOR(S)

8. Catherine Delea and Sarah Lerner
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Studies of the role of adrenal cortical steroids in the sodium retention of the edematous state.

Objectives: The objectives of this study are to elucidate the mechanisms whereby sodium and water homeostasis is normally maintained and to investigate the pathologic physiology of salt and water retention in edema formation.

Methods Employed: a. In metabolic balance studies, normal subjects were subjected to various experimental conditions and sodium potassium balance and urinary aldosterone were studied.
b. Similar studies were carried out in subjects with pathologic sodium retention.
c. Renal clearances were determined in patients with Addison's disease to determine the acute effects of aldosterone on electrolyte transport.

Patient Material:

		No.	Average stay days
Admissions:	Adult males	10	10
	Adult females	6	20

Major Findings: Three types of stimuli were found to influence aldosterone secretion, to wit, (a) ACTH, (b) changes in dietary potassium (c) changes in extracellular fluid volume.

a. A biphasic response to ACTH was observed. ACTH produced in all cases, a rise of aldosterone secretion. When it was continued long enough, there ensued a fall to levels lower than those before ACTH was started.

b. Potassium loading was found to produce rises in aldosterone excretion even when sodium loss was prevented by silultaneous sodium administration. Potassium deprivation was followed by a fall of aldosterone secretion. Such deprivation was accompanied by marked sodium retention.

c. Contraction of ECF volume was consistently effective in producing rises of aldosterone secretion and expansion of ECF volume consistently produced a fall when aldosterone excretion had first been elevated by contraction of ECF or by disease states.

Significance to Heart Research: It has been shown repeatedly that the salt retention in edematous states including cardiac failure is accompanied by high levels of urinary aldosterone. It is clearly of importance in determining the physiologic significance of edema and the fundamental mechanism by which it is produced to understand the normal mechanisms whereby aldosterone secretion is controlled.

Proposed Course: a. Studies are in progress to elucidate the biphasic response to ACTH by attempting to reproduce, and to suppress it with the use of DOCA and delta-1 fluorohydrocortisone.

b. The effect of potassium loading and deprivation on inulin space is being studied to elucidate the relationship of volume changes to potassium effects.

c. The effect of changes of the intravascular volume and aldosterone secretion are being studied with the use of auto-transfusion and albumin.

Analysis of NIH Program Activities

Budget Data

10. NHI-156C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications

15. NHI-156C
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

3. Clinical Endocrinology
SECTION

5. NHI-157C
SERIAL NO.

6. Cellular Transport Systems
PROJECT TITLE

7. A. Despopoulos
PRINCIPAL INVESTIGATOR(S)

8. Mrs. John Redd
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Cellular transport mechanisms

Objectives: To describe the reactions by which tissues can accumulate the specific substances required for synthesis of biologically required materials and eliminate the unessential products of cellular metabolism.

Methods Employed: The general objectives described are approached specifically by the examination of renal function in patients, in intact animals and in tissue experiments. The data derived from in vitro experiments are correlated with studies in patients and in intact animals.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	1	2
	Adult females	3	6

Major Findings:

1. Probenecid, a compound described as increasing the excretion of phosphorus in hypoparathyroid patients, was without effect in the patients examined.
2. 5-hydroxyindole acetic acid, the end product of tryptophan metabolism, is excreted actively by the kidney.
3. Uric acid, barbituric acid and their analogues inhibit active accumulation of p-aminohippuric acid in kidney slices.

Proposed course of project: A series of compounds will be tested by the tissue method in order to correlate their chemical characteristics with their affinity for transport by the kidney. Whenever practical, these compounds will also be examined in intact animals. Inhibitors of the transport system will be examined to locate insofar as is possible their locus of action.

Significance to Heart Research: An understanding of the renal excretory mechanisms may make it possible to design drugs which will hasten the excretion of undesirable body constituents or which will retard the excretion of desirable materials. An extension of this work to body tissues in general may ultimately permit an understanding of the mode of action of drugs and of the mechanisms responsible for the maintenance of tissue integrity.

Analysis of NHI Program Activities

Budget Data

10. NHI-157C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications

15. NHI-157C
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. In vitro effects of acetate ion on renal metabolism of p-amino-hippurate (Am. J. Physiol - in press)

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

5. NHI-158C
SERIAL NO.

6. Dynamics of Aortic Stenosis
PROJECT TITLE

7. Dr. Harold T. Dodge - Dr. Herbert L. Tanenbaum
PRINCIPAL INVESTIGATOR(S)

8. Miss Clara V. King
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Development of methods to further evaluate the dynamics of aortic stenosis.

Objective: A better definition of the pathologic physiology in the patient with aortic stenosis.

Methods Employed: In conjunction with the surgical group who have developed methods for measuring left intraventricular and aortic pressures, we have been doing cardiac outputs by means of the dye method, at first using a multiple sampling device with analysis of individual timed dye containing blood samples.

Patient Material:

	No.	Average Stay Days
Admissions: Adult males	3	21

Major Findings: After a period of trial and error concerning site of dye injection, method of sampling and analysis of curves, etc., we are finally in a position to start collecting data.

Significance to Heart Research: These studies will make it possible to calculate not only the pressure gradient across the aortic valve, but also left ventricular work, effective left ventricular work, aortic valve resistance and to approximate the aortic valve orifice size. The data can also then be compared to post operative data.

Proposed Course of Project: Work is now in progress to record outputs by means of a cuvette and densitometer with direct recording of the dye curves. Also further studies on methods to inject and measure dye directly into the left atrium at the time of bronchoscopy and left atrial puncture.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-158C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956:

Surgery Branch - National Institutes of Health - National Heart Institute.

14. None

Honors, Awards, and Publications Sheet

15. NHI-158C
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

5. NHI-159C
SERIAL NO.

6. Study of Role of Adrenal in Edema Formation
PROJECT TITLE

7. Leroy E. Duncan, Jr., M.D.
PRINCIPAL INVESTIGATORS

8. Grant W. Liddle, M.D., Mrs. Katherin Buck
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: The study of the role of the adrenal hormone, aldosterone, in the abnormal retention of salt and water which causes edema in heart disease and liver disease.

Objectives: A better understanding of the mechanism of edema formation.

Methods Employed: Clinical methods, metabolic ward technique including preparation of constant diets, analytical chamical technique, bioassay technique.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	9	46
	Adult females	2	100

Major Findings: It has been shown that the adrenal response to the accumulation of salt and water in the body is qualitatively normal but quantitatively grossly inadequate in patients with severe heart disease. This inadequate response is an important factor in the formation of edema.

Significance with Respect to Heart Disease: An improved understanding of the mechanisms involved in heart failure will lead to improved treatment of this condition.

Proposed Course of Project: No further experiments on this problem are planned by this investigator for the immediate future.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-159C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Analysis of NIH Program Activities

Honors, Awards, and Publications

15. NHI-159C
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

5. NHI-160
SERIAL NO.

6. Study of filtration of solutes into intima of aorta
PROJECT TITLE

7. Leroy E. Duncan, Jr., M. D.
PRINCIPAL INVESTIGATOR(S)

8. Mrs. Katherin Buck, Mr. Alfred Casper
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Study of filtration of solutes into intima of aorta.

Objective: A better understanding of the factors which modify the movement of various sized molecules from the blood stream into the wall of the aorta.

Methods employed: The solute to be studied is injected into a series of animals. At desired times the animals are killed and the concentration of the test solute in the serum and in the inner half of the aortic wall determined.

Major Findings: The test method has been worked out and the rate of movement of sucrose and inulin into the intima studied.

Significance to Heart Research: Information on factors which affect the rate of movement of various sized molecules into the aortic intima may be pertinent to the movement of lipoproteins into the aortic intima in atherosclerosis.

Proposed course of project: The rate of movement of larger molecules will be studied next. Following that the effect of various factors (hormonal influences, etc.) on the rate of movement of solutes into aortic intima will be studied.

Analysis of NIH Program Activities

Budget Data

10. NHI-160
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications

15. NHI-160
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

2. Clinical Investigations
SECTION

5. NHI-161
SERIAL NO.

6. The Evaluation of the Mechanical and Hydrodynamic Characteristics of the Cardiovascular System in Animals
PROJECT TITLE

7. Donald L. Fry, M. D.
INVESTIGATOR (S)

8. Frank J. Noble, B.E.E., Alexander J. Mallos, B.S., Alfred Casper, B.A., Raymond Kelly, Harold C. Smith, Jr.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: It was the original aim of this study to evaluate the power output of the heart only. The techniques devised to do this however opened up a new approach to the entire field of cardiovascular mechanics and hemodynamics. It is proposed now to expand the original study of heart power output to include the measurement of:

1) the viscous and elastic properties of the vascular bed and aorta in vivo,
2) the measurement of the transmission time of the pulse wave between the various points of the vascular beds
3) the dynamic dimensions of the aorta in vivo
4) the mean pressure throughout the vascular bed
5) the phase and group velocity of the pressure pulse wave
6) the ejection curve of the heart
7) the flow curve in the distal aorta

Objectives: On the basis of theoretical considerations, the above measurements should lead to: 1) the power output of the heart 2) the power losses in the aorta and peripheral vascular bed 3) elucidation of the "standing waves" mechanism 4) elucidation of the role of elasticity and viscosity in the normal function of the vascular system 5) prediction of the ejection curve from the peripheral pressure pulse.

Analysis of NIH Program Activities

Project Description Sheet

Methods employed: Blood velocity is measured by means of a new type flow-meter developed for this study and which is inserted directly in the aortic arch. Aortic pressure is measured by means of a short flexible trocar inserted in the aorta. Instantaneous aortic diameter is measured by electronic calipers developed for this project. A Survey Gage is utilized to measure pressures in the arteries at various points in the vascular system. By mathematical consideration of the above measurements, we hope to attain the objectives stated.

Major Findings: 1) A new type of plastic blood flowmeter has been developed which does not alter the physiological state of the animal radically when it is used. 2) It has been established that the mean pressure varies only slightly between the aortic valves and the femoral arteries although marked changes in contour occur.

Significance to Heart Research: The development of an accurate method for measuring instantaneous cardiac output would make possible the evaluation of pharmacologic agents and surgical procedures in the treatment of heart and vascular disease. A broader knowledge of the mechanical and hydrodynamic principles of the cardiovascular system might lead to a greater insight into the etiology and treatment of cardiovascular disorders.

Proposed course of project: Current research is directed along avenues leading to the simultaneous measurement of all parameters outlined above.

Analysis of NIH Program Activities

Budget Data

10. NHI-161
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE OR OTHER ORGANIZATIONS PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Laboratory of Technical Development, National Heart Institute, National Institutes of Health

Honors, Awards, and Publications

15. NHI-161
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

5. NHI-162C
SERIAL NO.

6. Hemodynamic Factors Governing the Size of the Left Atrium in Mitral Regurgitation
PROJECT TITLE

7. Robert P. Grant, M.D., Andrew G. Morrow, M.D., Herbert Tanenbaum, M.D.
PRINCIPAL INVESTIGATOR(S)

8. Miss Clara V. King, Miss Estelle Resnick
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: In mitral valve disease there is an astonishing variation in the size of the left atrium. Some of the factors underlying this variation are being studied.

Methods Employed: Rapid bi-plane angiocardiography makes it possible to measure the volume of the left atrium; the bronchoscopic technique for left heart catheterization makes it possible to measure the pressure variations in the left atrium; the "blue dye" method is used for determining cardiac output. Anatomical studies of the heart with mitral valve disease have been preciously published by Dr. Grant and form the basis for the interpretation of the data, from an anatomical point of view. Over sixty cases of mitral stenosis and insufficiency have been studied by these methods so far, and there are as many cases of other types of heart disease studied by the same methods which serve as the "controls".

Patient Material:

		No.	Average Stay Days
Admissions:	Adult Males	32	22
	Adult Females	25	20
Outpatient:	Number of patients	10	
	Number of visits	18	

Major Findings: When the rheumatic mitral valve deformity is associated with insufficiency of a marked degree as indicated in the pressure curves, the left atrium is always in excess of 800 cc. in volume. We have four cases in which the left atrial volume exceeds one and a half liters. If the mitral insufficiency is due to non-rheumatic disease (ruptured chorda tendinae, for example), the same degree of regurgitation is not associated with nearly as striking left atrial dilatation. From the studies the clinical features of the "giant left atrial syndrome" are emerging.

Analysis of NIH Program Activities

Project Description Sheet

NHI-162C
SERIAL NO.

Significance: It is believed that the unusual type of left atrial enlargement which is found with rheumatic mitral regurgitation is due to myocarditic involvement of the left ventricle and left atrium. The studies of pathologic material confirm this by demonstrating actual atrophy of certain regions of the left ventricle in such cases. The clinical picture in marked mitral regurgitation of the giant left atrium type is different in many ways from that of conventional mitral stenosis, and this clinical picture has been clarified by these studies.

Proposed Course: A more dynamic approach to this syndrome is now appropriate. We are planning to study the effects on left atrial and left ventricular function of rapid saline infusions and of graded exercise. Animals with experimental mitral regurgitation will be studied also.

Analysis of NIH Program Activities

Budget Data

10. NHI-162-C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications

15. NHI-162C
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

5. NHI-163
SERIAL NO.

6. The Evaluation of Cardiac Reserve in Human Subjects as Reflected by Alterations in Lung Elasticity and Resistance to Gas Flow
PROJECT TITLE

7. Donald L. Fry, M.D.
PRINCIPAL INVESTIGATOR(S)

8. Robert Hyatt, M.D., Charles McCall, M.D., Alexander Mallos, B.S.
OTHER INVESTIGATORS Harriette Coburn and Raymond Kelly

9. PROJECT DESCRIPTION

Project: To evaluate the cardiac reserve in human beings as reflected by the alterations in lung elasticity and resistance to gas flow.

Objectives: Previous studies have shown that the elastic properties of the lung and the resistance to gas flow are markedly altered in cardiac decompensation. The purpose of this study is to determine at what level of "cardiac reserve" these changes become apparent. It is hoped that these alterations of pulmonary function may reflect embarrassment of cardiac reserve not detectable by conventional means.

Methods Employed: The intraesophageal pressure recording balloon method was used to measure the elastic properties of the lung. Other procedures as outlined by D.L. Fry et al. The mechanics of Pulmonary Ventilation in Normal and in Patients with Emphysema. Amer. J. Med. XVI:80 1954.

Major Findings: Working in conjunction with the National Instrument Laboratories an electromechanical system was devised to maintain constant gas volume in a patient spirometer system. A thermal conductivity cell senses the concentration of a tracer gas, helium. Any change in concentration is thereby converted to an electrical impulse through a servo-mechanism which admits oxygen to the spirometer. In this way the metabolic needs of the subject are constantly met in spite of wide variations in oxygen consumption.

Significance to Heart Research: The possible development of a method for early detection of reduced cardiac reserve.

Proposed Course of Project: Considerable difficulty has been met in perfecting the constant gas volume system. The injecting mechanism tends to "hunt". Present efforts are toward perfection of this device for patient use.

Analysis of NIH Program Activities

BUDGET DATA

10. NHI-163
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. None

14. None

Honors, Awards, and Publications

15. NHI-163
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

5. NHI-164
SERIAL NO.

6. Evaluation of the Recording Characteristics of Modern Physiological Pressure Measureing Systems
PROJECT TITLE

7. Donald L. Fry, M.D.
PRINCIPAL INVESTIGATOR(S)

8. Frank W. Noble, M.E.E., Alexander Mallos, B.S., Robert Gorman, Alfred Casper
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: The evaluation of the recording characteristics of commercially available physiological pressure recording systems.

Objectives: A comprehensive evaluation of the stability, static accuracy and dynamic accuracy of the many combinations of catheters and physiological pressure gages that are commonly used. To make available to the investigator pressure gages that are commonly used. To make available to the investigator a fund of reference data from which he may choose a pressure recording system for his specific study which will reduce recording artefacts to a minimum.

Methods Employed: Established methods and techniques for measuring static response, dynamic response, and stability were employed.

Major Findings: High fidelity pressure pulse tracings cannot be recorded via conventional catheter-manometer systems, although they are adequate for measuring mean pressures. When high frequency response is required, a gage attached directly to a needle or a catheter tip manometer should be used. Gages and catheter systems varied widely in the qualities for which they were evaluated. Care must be exercised to select the proper system for the particular study.

Significance to Heart Research: Data have been compiled and presented to the investigator who is interested in accurate measurement of pressure events. Although pressure manometer systems are in common use in connection with cardiac catheterization and other laboratory procedures, these limitations are little understood. A knowledge of the characteristics of his measuring instruments, should enable the investigator to obtain more faithful pressure contours and more confidently interpret his experimental data.

Proposed Course of Project: Preparation of the manuscript for publication.

Analysis of NIH Program Activities

Budget Data

10. NHI-164
SERIAL NO.

12. BUDGET ACTIVITY: Research

13. None

14. None

Honors, Awards, and Publications

15. NHI-164
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

5. NHI-165C
SERIAL NO.

6. The Effects of Reserpine and Chlorpromazine on Gastric Secretion and Intestinal Motility
PROJECT TITLE

7. Bernard J. Haverback, M.D.
PRINCIPAL INVESTIGATOR(S)

8. Albert Sjoerdsma, M.D., Roger McDonald, M.D., Luther L. Terry, M.D.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Determination of the effects of two hypotensive drugs, reserpine and chlorpromazine, on gastric secretion and intestinal motility in man.

Objectives: The objective of this project is to determine the effects of reserpine and chlorpromazine on the volume, acidity, and pepsin content of gastric secretion and on intestinal motility.

Methods Employed: Continuous gastric aspiration is accomplished by means of a mechanical suction pump. Intestinal motility is recorded by means of an open-tip intestinal tube connected to a pressure transducer.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	10	4
	Adult females	10	4

Major Findings: Reserpine increases the volume and acidity of gastric secretion. Chlorpromazine decreases the volume of gastric secretion without significantly affecting the acidity. Preliminary studies indicate that reserpine does not alter uropepsin excretion.

Significance to Heart Research: Two hypotensive drugs have been shown to have opposite effects on gastric secretion. Because reserpine stimulates gastric acid secretion, it should be used cautiously in patients with peptic ulceration.

Proposed Course of Project: Further studies are in progress to determine the effects of reserpine on gastric pepsin, blood pepsinogen, and uropepsin.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-165C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956.

National Institute of Mental Health
Roger McDonald, M.D.

14. None

Analysis of NHI Program Activities

Honors, Awards, and Publications

15. NHI-165C
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

The Effects of Reserpine and Chlorpromazine on Gastric Secretion: Haverback, B.J., Stevenson, T.D., Sjoerdsma, A., and Terry, L.L. Am. J. Med. Sc.: 230,601, 1955.

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

5. NHI-166C
SERIAL NO.

6. Interrelationships Between Reserpine and Serotonin in Man
PROJECT TITLE

7. Bernard J. Haverback, M.D.
PRINCIPAL INVESTIGATOR(S)

8. Parkhurst Shore, Ph.D., Edward Tomich, M.S., Bernard Brodie, Ph.D. Thomas Dutcher, M.D., Luther L. Terry, M.D.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Interrelationship Between Reserpine and Serotonin in Man

Objectives: The objective of this project is to determine the cumulative effect of small doses of reserpine on serotonin in man.

Methods Employed: Reserpine was administered to hypertensive patients and platelet serotonin was determined during and following cessation of reserpine administration. The subjects are patients who are being studied for hypertension.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	4	30
	Adult females	1	30
Outpatient:	Number of patients	5	
	Number of visits	10	

Major Findings: Reserpine in dosage of 1 mgm daily reduced platelet serotonin levels approximately 95% in 10 days. No detectable levels of serotonin were found in platelets of hypertensive patients who had been on oral reserpine for 3 months. The absence of platelet serotonin had no effect on hemostasis.

Significance to Heart Research: As reserpine is a drug widely used in the treatment of hypertension, an eludication of its mechanism of action is of interest.

Proposed Course of Project: An extension of the studies of the cumulative action of reserpine on serotonin in man is planned. Also studies to elucidate the effects of the altered serotonin metabolism on gastric secretion and urinary sodium excretion is planned.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-166C

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN FISCAL 1955 or 1956.

Laboratory of Chemical Pharmacology
Parkhurst Shore, Ph. D.
Edward Tomich, M.S.
Bernard Brodie, Ph.D.

Clinical Pathology
Thomas Dutcher, M.D.

14. None

Honors, Awards, and Publications

15. NHI-166C
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

5. NHI-167
SERIAL NO.

6. The Effects of Serotonin on Gastric Secretion and Intestinal Motility
PROJECT TITLE

7. Bernard J. Haverback, M.D.
PRINCIPAL INVESTIGATOR(S)

8. Adrian Hogben, M.D., Neil Moran, M.D., Luther L. Terry, M.D.
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Determination of the effects of serotonin on gastric secretion and intestinal motility in the dog.

Objectives: The objective of this project is to determine the effects of serotonin on gastric secretion and intestinal motility and to evaluate serotonin antagonists.

Methods Employed: Serotonin was administered intravenously and gastric secretion was obtained from dogs with Heidenhain pouches and from dogs with intact stomachs. Intestinal motility was recorded from the jejunum by means of an open-tip intestinal tube connected to a pressure transducer.

Major Findings: Serotonin did not change gastric secretion in a clear cut manner, but it appears that in low dosage serotonin is a mild stimulus to gastric secretion, but at higher dosage, gastric secretion is inhibited. Serotonin is a potent stimulus to intestinal motility and is not antagonized by atropine or hexamethonium. Lysergic acid diethylamide, a presumed serotonin antagonist, potentiated the stimulus of serotonin to intestinal motility.

Significance to Heart Research: Serotonin has been implicated in valvular heart disease in the malignant carcinoid syndrome. In addition to elucidating the effects of serotonin on the gastrointestinal tract, the present studies make available an *in vivo* method to evaluate serotonin antagonists.

Proposed Course of Project: Evaluation of new serotonin antagonists by this method is planned.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-167
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honors, Awards, and Publications

15. NHI-167
SERIAL NO.

16. None

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

5. NHI-168C
SERIAL NO.

6. The Role of the Adrenocortical Steroids in the Salt Retention of the Edematous States.
PROJECT TITLE

7. Grant W. Liddle, M.D.
PRINCIPAL INVESTIGATOR(S)

8. Frederic C. Bartter, M.D., Leroy E. Duncan, M.D., June Richard, Gaynelle Greene, Catherine Delea, Katherin Buck, Alfred Casper, Walter Gray
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: The role of the adrenocortical steroids in the salt retention of the edematous states.

Objectives: To evaluate the role of the adrenal cortex in regulating salt excretion in health and disease. Special attention is directed toward understanding the mechanisms regulating the secretion of the most potent of the natural salt-retaining steroids - aldosterone. The ultimate hope is that of learning why aldosterone secretion is excessive in patients with congestive heart failure.

Methods Employed: The key procedure is the measurement of the amount of aldosterone appearing in the urine of patients under various experimental conditions. The patients are generally maintained on a "metabolic balance regimen." The aldosterone is extracted from the urine with suitable organic solvents, and when appropriate, it is farther purified by chromatography. The amount of aldosterone in the purified extract is then estimated by observing its effects on electrolyte excretion in the adrenalectomized dog (method of Liddle, Cornfield, Casper, and Bartter)

Patient Material: Approximately 40 subjects (half of them normal volunteers) have been studied for periods varying from a few days to a number of months. The patients whose disease processes made them especially suitable for these investigations included several with congestive heart failure, and some with cirrhosis, nephrosis, Addison's disease, hypopituitarism, mammary carcinoma (treated with adrenal blocking agents) hypertension, neurogenic hypotension, pulmonary carcinoma (with hyponatremia), renal insufficiency (with hyperkaliemia) and diabetes insipidus.

Analysis of NIH Program Activities

Project Description Sheet

NHI-168C
SERIAL NO.

Patient Material continued:

		No.	Average Stay Days
Admissions:	Adult Males	30	43
	Adult females	9	24

Outpatient:	Number of patients	5
	Number of visits	15

Major Findings: (A) Aldosterone secretion is an inverse function of extracellular fluid volume. It is, therefore, extremely responsive to changes in sodium balance. (B) Aldosterone secretion is increased slightly and transiently by administration of ACTH, but the latter is clearly not as important as water and electrolyte factors in regulating aldosterone secretion. (C) Suppression of ACTH secretion by administration of cortisone and hydrocortisone in large amounts does not affect aldosterone secretion to any marked degree. (D) Modification of aldosterone can be interpreted best not as a specific effect of sodium, but rather as an effect of the water which moves with sodium in expanding or contracting extracellular fluid volume. (E) Although the secretion of aldosterone is excessive in congestive heart failure and in cirrhosis, still the aldosterone-producing mechanism responds in a qualitatively normal fashion to changes in sodium (and extracellular water) balance in these patients.

Significance to Heart Research: Abnormal accumulation of sodium, and therefore water, is one of the most troublesome aspects of congestive heart failure.

It appears that excessive secretion of aldosterone is one of the essential links in the chain of events leading to this accumulation of fluid. We feel that it is important, therefore, to learn why aldosterone secretion is excessive in congestive heart failure with the ultimate hope that this will lead to more intelligent management of such problems.

Proposed Course of Project: To determine by what mechanism changes in extracellular fluid volume result in changes in aldosterone secretion and to determine how this mechanism becomes disturbed in those diseases characterized by abnormal accumulation of fluid.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-168C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OT OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956.

1. Mr. Jerome Cornfield of the Office of Biometry performed extremely valuable service in working out the statistical analysis of our aldosterone assay method.

2. Dr. Roy Hertz of NCI has provided specimens from patients receiving an adrenal blocking agent, "Amphenone B".

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Dr. James O. Davis of NHI has carried out in dogs experiments somewhat similar to our clinical studies, attempting to idlentify the role of aldosterone in experimental congestive heart failure.

Honors, Awards, and Publications

15. NHI-168C
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Liddle, G.W., Cornfield, J., Casper, A.G.T., and Bartter, F.C.: The Physiological Basis for a Method of Assaying Aldosterone in Extracts of Human Urine. Journal of Clinical Investigation: 34:1410, September, 1955.

2. Liddle, G.W., Duncan, L.E., Jr., and Bartter, F.C.: Dual Mechanism Regulating Adrenocortical Function in Man. American Journal of Medicine (in press).

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

5. NHI-169C
SERIAL NO:

6. Pharmacological Studies with Newer Synthetic Adrenocortical Steroid Analogs.
PROJECT TITLE

7. Grant W. Liddle, M.D.
PRINCIPAL INVESTIGATOR(S)

8. June F. Richard and Gaynelle Greene
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Pharmacological studies with newer synthetic adrenocortical steroid analogs.

Objective: To understnad the physiologic effects of the newer synthetic steroid analogs with the dual purpose of finding better therapeutic tools and of gaining additional insight into the relationship between structure and function of the steroids.

Methods Employed: The actions of several new steroids have been observed in a series of appropriate patients maintained, generally, on a metabolic balance regimen. Preliminary and parallel studies have often been carried out in adrenalectomized dogs as well. The effects of new steroids on electrolyte excretion, nitrogen balance, and on the spontaneous secretion of adrenocortical hormones have been observed. The latter have been measured by a variety of recently developed chemical and biological procedures.

Patient Material: Approximately 16 patients, half of them normal volunteers have been employed for periods ranging from one to three months. The patients whose disease processes made them suitable for these investigations included one with hyperpituitarism, four with Addison's disease, one with suspected adrenocortical tumor, one with adrenal hyperplasia with sodium wasting and masculinization, two with nephrosis, and two with cirrhosis.

		No.	Days
Admissions:	Adult males	10	34
	Adult females	3	20

Analysis of NIH Program Activities

Project Description Sheet

NHI-169C
SERIAL NO.

Major Findings: Delta-1, 9-alpha fluorohydrocortisone is the most potent ACTH-suppressing steroid yet produced. It has been employed in a number of diagnostic studies and in a series of pharmacological studies in which it was desirable to clear the blood and urine of 17-hydroxycorticoids. In the latter studies it was possible to show that considerable quantities of hydrocortisone are absorbed into the systemic circulation after topical application to rectal or vaginal mucous membranes - an observation of some potential interest since the topical one of such steroids is becoming a fairly widespread practice.

A new series of corticosteroid analogs, the 2-methylated steroids, has been available and we have found that the introduction of the 2-methyl group enhances both the glucocorticoid and the salt-retaining activities of the hydro-cortisones. Salt-retaining activity is enhanced out of proportion to the degree of enhancement of glucocorticoid activity. In fact, 2-methyl, 9-alpha fluorohydrocortisone has been found to be 3 times as potent as aldosterone in salt-retaining activity and is, therefore, the most potent such steroid known at the present time. An interesting feature of the 2-methy hydrocortisones is that they have prolonged action. After single oral or intravenous doses are given, the metabolic effects can be observed for about 48 hours. This may be related to the fact that the methylated steroid is not metabolized to inactive form as rapidly as is the non-methylated analog.

Significance to Heart Research: This is largely indirect. Many diseases affecting the heart and blood vessels are themselves affected by adrenal steroids. It is expected that a greater knowledge of the functions of a variety of steroids may increase our understanding of how such steroids as aldosterone and hydrocortisone affect the cardiovascular system - either as therapeutic agents or as pathogenic factors.

Proposed Course of Project: To study the physiologic actions of additional members of the 2-methylated series of steroids in order to correlate further the structure and function of such compounds.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-169C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR PROJECT IN EITHER 1955 or 1956:

1. Synthetic steroids have been provided by the following pharmaceutical houses:

Upjohn
Squibb
CIBA

2. Dr. Ralph Peterson of NIAMD has provided valuable advice regarding chemical procedures employed in steroid determinations.

14. None

Honors Awards and Publications

15. NHI-169C
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Liddle, G.W., Richard, J.E., and Peterson, R.E.: An Improved Method for Assaying the Steroidogenic Potency of ACTH. Endocrinology 57:594, November, 1955.

2. Liddle, G.W. and Richard, J.E.: The 2-Methyl Hydrocortisones: A New Series of Steroids with Enhanced Potency and Prolonged Action. Science (in press)

3. Liddle, G.W.: Delta-1, 9-alpha-Fluorohydrocortisone: A New Investigative Tool in Adrenal Physiology. J. Clin. Endocrinology & Metabolism (in press).

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

5. NHI-170C
SERIAL NO.

6. Studies on Vasoactive Substances
PROJECT TITLE

7. Albert Sjoerdsma, M.D., Ph.D.
PRINCIPAL INVESTIGATOR(S)

8. J. D. Davidson L. L. Terry B. J. Haverback, T. P. Waalkes
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Studies on vasoactive substances.

Objectives: Relate naturally occurring vasoactive substances to cardiovascular disease and response to drugs.

Methods employed: Using chemical methods, the metabolism of serotonin (5-hydroxytryptamine) was studied in vitro and in 7 patients with the recently described syndrome of malignant carcinoid tumors with valvular heart disease, asthma, cutaneous flushing and diarrhea. Using a fluorescence method, plasma epinephrine and norepinephrine levels were measured in normals and in patients with pheochromocytoma. Radioactive precursors of serotonin and epinephrine were administered to some patients and radioactive products in the urine analyses. A metabolic balance study was done on one of the carcinoid patients and three of these patients were subjected to cardiac catheterization.

Major Findings:
1) Malignant Carcinoid Syndrome - Blood serotonin levels were shown to range from 0.5 to 2.7 µgm/ml compared to normal values of 0.1 to 0.3 µgm/ml. Analyses of several carcinoid tumors showed a serotonin content of 1 to 30 mgm/gm. and the presence of enzymes required to form and destroy serotonin. Urinary excretion of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA) ranged from 76 to 580 mgm/day in carcinoid patients as compared to 2 - 9 mgm for normals and patients with other conditions. The elevation of urinary 5-HIAA affords a simple and rapid means of diagnosis. The precursor relationship of tryptophan to 5-hydroxyindoles in man was demonstrated by a metabolic balance study and tracer studies on these patients. The manifestations of the carcinoid syndrome are apparently the result of serotonin excess but an alteration of tryptophan metabolism with disturbance in niacin and protein must also be considered in pathogenesis.

2) Pheochromocytoma: Five patients with this disorder have been diagnosed chemically. Administration of a C^{14} precursor of epinephrine to one of these patients revealed a radioactive product in one urine which has not been characterized chemically but which may be of importance diagnostically.

Significance to Heart Research: Chiefly in the fact that we are dealing with 2 conditions in which the abnormality consists of the over-production of naturally occurring substances, one leading to valvular heart disease, the other to hypertension.

Proposed course of project: Attempt to 1) produce valvular heart disease in animals by chemical means and 2) study further the metabolism of epinephrine in patients with pheochromocytoma in the hope of developing a simpler means of diagnosis.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	10	60
	Adult females	5	60
	Children female	1	20
Outpatient:	Number of patients	50	
	Number of visits	90	

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-170C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

1. National Heart Institute, Laboratory of Chemical Pharmacology
2. Dr. Thomas Mattingly, Walter Reed Army Hospital, Washington, D. C.
3. Dr. William Manion, Armed Forces Institute of Pathology, Washington, D. C.
4. Naval Medical Center, Bethesda, Md.

Provided cooperation and patient material but not actual support.

14. IF THIS PROJECT RESEMBLES COMPLEMENTS OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL FACILITIES OR FUNDS) IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

Drs. McDonald and Everts - National Institute of Mental Health

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. NHI-170C
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. A. Sjoerdsma, T. E. Smith, T. D. Stevenson and S. Udenfriend: Metabolism of 5-hydroxytryptamine by monoamine oxidase. Proc. Soc. Exper. Biol. & Med. 89:36,1955.

2. Sjoerdsma, A., Weissbach, H. & Udenfriend, S.: Simple Test for Diagnosis of Metastatic Carcinoid. J.A.M.A. 159:397,1955.

3. Sjoerdsma, A., Terry, L.L., and Udenfriend, S.: Malignant Carcinoid, a New Metabolic Disorder. Monograph related to Exhibit. NHI, October, 1955.

4. Sjoerdsma, A., Weissbach, H., and Udenfriend, S.: A Clinical, Physiologic and Biochemical Study of Patients with Malignant Carcinoid (Argentaffinoma). Am.J.Med. In Press.

5. Udenfriend, S., Weissbach, H., and Sjoerdsma, A.: Studies on Tryptophan and Serotonin in Patients with Malignant Carcinoid. Science. In Press.

6. Haverback, B.J. and Sjoerdsma, A. and Terry, L.L.: Urinary Excretion of Serotonin Metabolite in Various Clinical Conditions. In Preparation.

7. Sjoerdsma, A., Kornetsky, C.H. and Everts E.: Effect of LSD in Patients with Excess Serotonin. Submitted for publication.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therpaeutics

5. NHI-171C
SERIAL NO.

6. Study of Drugs Affecting the Cardiovascular System

7. Albert Sjoerdsma, M.D., Ph.D., & John D. Davidson, M.D.
PRINCIPAL INVESTIGATOR(S)

8. Bernard J. Haverback, M.D., Luther L. Terry, M.D., T. Phillip Waalkes, M.D., and Mr. William King
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Study of Drugs Affecting the Cardiovascular System

Objectibes: To study the metabolism and pharmacologic effects of various agents in man.

Methods & Materials: Drugs are given both orally and intravenously to hypertensive and non-hypertensive individuals. In metabolic studied, measurements are made on blood and urine. Pharmacologic effects are judged by changes in several parameters including subjective reactions, blood pressure, pulse, respiration, pupil size, gastric secretion, intestinal motility, and vascular responsiveness.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	45	60
	Adult females	40	60
	Children mal	3	25
	Children female	3	25
Outpatient:	Number of patients	120	
	Number of visits	90	

Major findings: 1) Priscoline. This agent was discovered to induce cardiac pain in a patient with arteriosclerotic heart disease. Animal experiments (Pharmacology, NHI_ indicate that this effect is due to a sudden increase in cardiac work as measured by cardiac contractile force.

2) Clinical trails with Aranthol (2-methylamine-6-hydroxy-6-methylheptane. In animals, this sympathomimetic amine has the property of increasing myocardial contractile force without significant pressor or central excitatory effects. In man, up to 3.0 gm/day in divided dosage produced nervousness and palpitations and failed to benefit the signs and symptoms of congestive failure.

NHI-171C
SERIAL NO.

3) The Fate of Papaverine. A specific and sensitive method for the estimation of papaverine in biological material has been developed. Its metabolism has been studied in man and animals. The drug was shown to be cleaved to formaldehyde and a phenolic metabolite.

4) The Sulfoxide of Chlorpromazine: Preliminary studies reveal that the major metabolite of chlorpromazine also produces both sedative and hypotensive effects in man.

5) Ambonestyl (2-diethylaminoethylisonicotinamide) in Cardiac Arrhythmias: This pronestyl-like compound was tested in both animals and humans and found to be relabively ineffective and too toxic for clinical use. Its metabolism is similar to that of pronestyl (procaine amide)

Significance in Heart Research: This approach is directed toward the discovery of better therapeutic agents.

Proposed Course of Project: Further evaluation of chlorpromazine-sulfoxide and attempt to discover the metabolic products of papaverine.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-171C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956.

Ambonestyl and Priscoline Study - in cooperation with Laboratory of Chemical Pharmacology - National Heart Institute

Aranthol Study - in cooperation with George Washington University - Department of Cardiology - D.C. General Hospital, Washingtoh, D.C.

Sulfoxide of Chlorpromazine --in cooperation with Laboratory of Chemical Pharmacology - National Heart Institute

14. None

Honors, Awards, and Publications

15. NHI-171C
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. J. D. Davidson: Induction of Cardiac Pain by Orla Priscoline; Submitted for Publication.

2. A. Sjoerdsma, J. Axelrod, M. Shofer, W. King and J.D. Davidson: The Fate of Papaverine. In Preparation.

17. None

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

5. NHI-172C
SERIAL NO.

6. Studies in Hypertension
PROJECT TITLE

7. Dr. John D. Davidson
PRINCIPAL INVESTIGATOR(S)

8. Dr. Albert Sjoerdsma, Dr. Bernard Haverback, Dr. Luther L. Terry, Dr. Thomas P. Stevenson, Dr. Phillip Waalkes
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: To study pharmacologic aspects of hypertension and the effectiveness of new drugs, primarily developed in the NHI.

Methods employed: Intravenous infusions of various agents are given to patients with persistent hypertension.

Major Findings:

1. Andromedotoxin, a crystalline substance obtained from the roots of Rhododendron maximum, was shown to have actions in humans similar to that of the veratrum alkaloids. Erratic oral absorption and side effects which included EKG changes, paresthesiae, and vomiting limit the use of this material to treatment of hypertensive crises.
2. Gastrointestinal side effects of reserpine and chlorpromazine: Reserpine was found to increase the volume and free acidity of gastric secretion while chlorpromazine reduced the volume but did not affect the free acidity.
3. Interaction of Various Antihypertensive Drugs: Preliminary studies designed to quantitate the degree of potentiation exerted by reserpine on the action of commonly used antihypertensive drugs suggest a true potentiation of blood pressure lowering produced by hemamethonium, and a less striking effect in the case of apresoline, veratrum and chlorpromazine.
4. Clinical trials with "ormosia" (alkaloid extract from the tree - ormosia panamensis). At an intravenous dose of 250 µgm/kgm, no significant hypotensive effect has been observed. This dose is uniformly effective in animals (Moran and associates).

Analysis of NIH Program Activities

Project Description Sheet

Significance to Heart Research: The treatment of hypertension has been in the pharmacologic approach to blood pressure reduction.

Proposed course of project: To continue the drug interaction study. Ormosia evaluation and study of other agents as they become available.

Patient Material:

		No.	Average Stay Days
Admissions:	Adult males	6	60
	Adult females	12	50
Outpatient:	Number of patients	20	
	Number of visits	75	

Analysis of NIH Program Activities

Budget Data Sheet

10. NIH-172C
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES OR PERSONNEL FOR THIS PROJECT IN EITHER 1955 or 1956. IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM 1)

Andromedotoxin and ormosia - H, LCNP and H, LCP

Honors, Awards, and Publications

15. NIH-172C
SERIAL NO.

16. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. T. D. Stevenson, A. Sjoerdsma, and L.L. Terry. Pharmacologic Aspects of Hypertension. Military Medicine. 117:101, 1955.
2. B. J. Haverback, T. D. Stevenson, A. Sjoerdsma and L. L. Terry The Effects of Reserpine and Chlorpromazine in Gastric Secretion. Am. J. Med. Sc. 230:601, 1955.

Analysis of NIH Program Activities

Project Description Sheet

1. National Heart Institute
INSTITUTE

2. Clinic of General Medicine and Experimental Therapeutics
LABORATORY OR BRANCH

5. NHI-173
SERIAL NO.

6. Application of Vector Methods to Clinical Electrocardiography
PROJECT TITLE

7. Robert P. Grant and Harold T. Dodge
PRINCIPAL INVESTIGATOR(S)

8. Clara V King and Margaret Austin
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: Vector methods, which bring greater precision and detail to the interpretation of the electrocardiogram, have been used for the study of the changes in cardiac electrical activity which accompany myocardial infarction and ventricular conduction disturbances.

Methods Employed: Tracings have been obtained from a number of different hospitals throughout the country from cases where normal electrocardiograms were available prior to and, if possible, after the development of the particular electrocardiographic abnormality. Vector methods make possible a mathematical calculation of the type and degree of electrical alteration which has taken place. Over three hundred such cases have been collected. In addition, over 600 cases have been collected who died and had a complete autopsy within a month of their last electrocardiogram, permitting correlation of the pathologic alterations with the clinical and electrical findings.

Major Findings: Three types of electrocardiographic abnormality which are themselves diagnostic of myocardial infarction which had not been previously recognized have been discovered. In addition, certain fundamental observations concerning the mechanism and nature of Bundle Branch block and peri-infarction block have been made.

Significance to Heart Research: These studies will help to put diagnostic clinical electrocardiography on a more objective and accurate basis than has been the case heretofore. They improve and expand the criteria for the diagnosis of myocardial infarction.

Proposed Course of Project: At present, the analysis of the 600 cases with correlation to pathologic alterations is underway.

Analysis of NIH Program Activities

Budget Data Sheet

10. NHI-173
SERIAL NO.

12. BUDGET ACTIVITY: RESEARCH

13. None

14. None

Honros, Awards, and Publications

15. NHI-173
SERIAL NO.

16. LIST ANY PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. The Mechanism of Ventricular Conduction Defects in Man, Left Ventricular Conduction Defects. H.P. Grant and H.T. Dodge - Accepted for publication, American Journal of Medicine.

2. The Mechanism of Ventricular Conduction Defects in Man, Right Ventricular Conduction Defects. H.T. Dodge and H.P. Grant. Accepted for publication, American Journal of Medicine.

3. Mechanism of A-V Arrhythmias. H.P. Grant - Accepted for publication, American Journal of Medicine.

Analysis of NIH Program Activities

January-December 1955

Division of Biologics Standards

The program of the Division of Biologics Standards this year must be evaluated in terms of the effectiveness of its control of biological products rather than in terms of its research accomplishments.

Important events of the past six months include the change in status of the organization from the Laboratory of Biologics Control to the Division of Biologics Standards, an increase in staff to permit the development of a well-rounded poliomyelitis vaccine testing program, and the initiation of a research section capable of active participation in all facets of research concerning biological products.

The Division is now functioning on the basis of four laboratories: Viral Products, Bacterial Products, Blood and Blood Products, and Control Activities. Personnel has been increased from less than 40 persons to 114. The table of organization, approved and signed by the Director of NIH, allows for 36 additional staff members during the fiscal year.

The poliomyelitis vaccine testing program, which has been assigned to the Laboratory of Viral Products, consists of two parts--one devoted primarily to testing with tissue cultures and the other to testing with cortisone-treated monkeys. The latter operation is self-contained, while that relating to tissue culture is composed of 5 teams. A serological laboratory has been established to serve all

units within the Division. Its establishment will permit activation of a group to test the potency of poliomyelitis vaccines.

Although research so far has been directed entirely toward developmental aspects of control testing, plans for a basic research program, primarly concerned with poliomyelitis virus, are under way. The studies contemplated should be applicable to other virus problems. Some of the studies will deal with the kinetics and genetics of viral populations and the mode of action of virucidal agents. Such studies are essential to an effective biologics control program.

A great deal of time and energy during the past six months has been expended in services connected with the deliberations of the Technical Committee for Poliomyelitis Vaccine. The coordination of the Committee with the technical representatives of the manufacturers has kept both government and industry informed, and has increased confidence between the producers and the regulating agency.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Division of Biologics Standards — INSTITUTE
2. Control Activities Branch — LABORATORY OR BRANCH
3. Office of Chief — SECTION
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. DBS-1 — SERIAL NO.
6. Office of Chief, Control Activities Branch — PROJECT TITLE
7. W. G. Workman, Medical Director — PRINCIPAL INVESTIGATOR(S)
8. Assistant Chief proposed in Fiscal Year 1957 — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION

Scope of Activity

Under the Director, Division of Biologics Standards, this project is responsible for the area of activity dealing directly with licensed establishments in relation to the control of biologic products, with the immediate support of sections on reference standards, on control tests and on pyrogens.

The activities include.

(a) Determinations of eligibility of applications and of products for license, this being made on the basis of review of physical equipment, review of business integrity of management, review of scientific qualifications of personnel, and of review of evidence of safety, purity and potency of products in compliance with legal requirements.

(b) Supervision of inspections of licensed manufacturers of biological products and of establishments applying for license.

Project Description - Continued

(c) Review of protocols, tests and all other data relating to safety, purity and potency and the release of individual lots of biologic products for distribution.

(d) The establishment and distribution of physical biological standards.

(e) Review of requirements and Regulations now in effect for such constructive revision as is needed and development of requirements and Regulations required for new products.

(f) Maintenance of close working relations between this Branch and the other Laboratories of the Division in order to insure continuous knowledge of all activities and data needed for licensing, testing and release of biologic products.

(g) Serving on various committees such as those of the American Medical Association, World Health Organization, Unites States Pharmacopoeia, and other official and non-official agencies operating in fields of interest similar to or identical with that of this Branch.

(h) Cooperation with the Department of National Defense and other governmental agencies on problems relating to the control of biologic products.

(i) Maintenance of contact with foreign governments for interchange of information relating to the control of biologic products.

Significance to Public Health

Nearly every person in this country at some time receives a biological product in the course of routine immunization against one or more diseases. In addition, medical situations frequently arise in which biological products are administered for immunotherapy or for other reasons as in the case of blood transfusions.

Any failure of the products used to meet acceptable standards of safety and effectiveness may result in serious and even widespread difficulties in medical and public health practice.

Proposed Course of Project

It is proposed to continue the activities stated above in such a manner as to insure the quality of licensed products distributed for use.

Project Description - Continued

It is proposed to strengthen the Control Test Section by the addition of two higher grade professional positions, to improve the quality of routine testing being done, and to develop better methods of testing for safety and for potency.

The Reference Standards Section is inadequately staffed due mainly to reassignment of the chief of the section. His early replacement is required and it is proposed to add a lower grade professional assistant. The establishment and maintenance of physical standards are fundamental in providing a basis for determining the potency of those products to which potency tests are applicable.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. DBS-1
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	/_/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/
BIOLOGICS STANDARDS	/X/		

12. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM I)

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

Does not resemble, complement or parallel research done elsewhere in the Public Health Service.

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

14. DBS-1
SERIAL NO.

15. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

16. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities
Project Description Sheet

1. Division of Biologics Standards 2. Control Activities Branch
INSTITUTE LABORATORY OR BRANCH

3. Reference Standards Section 4. _____
SECTION LOCATION (IF OTHER THAN BETHESDA)

5. DBS-2
SERIAL NO.

6. Preparation, standardization and distribution of biological standards.
PROJECT TITLE

7. Vacant,
PRINCIPAL INVESTIGATOR(S)

8. Mr. R. P. Miller, Biologist GS-7 and vacancy Bacteriologist GS-7
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives

Since biological products cannot be standardized, with few exceptions, by chemical or physical means, recourse must be had to testing their effects in relation to physical reference preparations. It is the function of this project to provide these standard preparations for the use of manufacturers and others engaged in standardization. In this way uniformity of potency is established throughout the country. It is largely by the use of these standards that those portions of the biologics law relating to potency can be enforced. A great deal of painstaking and responsible work is necessary to insure that standards issued are satisfactory as official standards for this country and bear a known relation to International Standards.

Methods Employed

Old standards must be replaced as they become exhausted. Standards are required for new products and for certain of the older ones for which it has not been possible to prepare standards heretofore. This is dependent on increasing knowledge in a number of fields,

(Continued on attached sheet)

such as immunology, biology, biochemistry, and medicine. Important features of this work are the improvement of methods of storing and preserving standards, such as by freezing and drying so that their potency may be maintained, and to correlate those standards where possible with those established in other countries or with International Standards.

Proposed Course of Project

Due principally to lack of an active head of the project since the former chief was reassigned in September, 1955, this project is barely meeting part of the essential needs. It is proposed that the project be thoroughly reevaluated from the point of view (1) of replacing standards nearing depletion, (2) of reinvestigating certain standards now in use and (3) of establishing standards, a few of which are badly needed but not in existence. For example, although poliomyelitis vaccine has been used clinically for more than two years, there is still no reference vaccine preparation suitable for establishment as a national (or international) standard.

Standards and Reference Preparations

A culture collection of organisms used in the production and testing of biologic products is also maintained. Official standards and reference preparations now maintained are as follows:

Anti-A Blood Grouping Serum
Anti-B Blood Grouping Serum
Antimeningococcus Serum
Antipneumococcic Serums (I,II,III,V,VII,VIII)
Anti-Rh Typing Serum
Blood Group Substances A and B
Botulinus (A) Antitoxin
Botulinus (B) Antitoxin
Botulinus (C) Antitoxin
Botulinus (A) Toxin
Botulinus (B) Toxin
Botulinus (C) Toxin
Cholera Opacity Reference
Cholera Vaccine
Diphtheria Antitoxin
Diphtheria Antitoxin Flocculating Serum
Diphtheria Toxoid
Dysentery Antitoxin
Fibrinogen
H. Influenzae Antiserum
Histolyticus Antitoxin
Histolyticus Toxin
Histoplasmin

Standards and Reference Preparations, Cont'd.

Influenza Virus Vaccine for CCA Titration
Influenza Virus Vaccine for Potency
Ludox Nephelometry Standard
Mumps Vaccine
Nitrogen Standard (Protein)
Oedematiens Antitoxin
Oedematiens Toxin
Old Tuberculin
Perfringens Antitoxin
Perfringens Toxin
Pertussis Turbidity
Pertussis Vaccine
Plague Agglutinating Serum
Plague Vaccine
Poliomyelitis Control Serum
Poliomyelitis Immune Globulin
Poliomyelitis Virus
Purified Protein Derivative of Tuberculin
Rabies Challenge Virus
Rabies Vaccine
Scarlet Fever Streptococcus Antitoxin
Scarlet Fever Streptococcus Toxin
Smallpox Vaccine
Sordellii Antitoxin
Sordellii Toxin
Staphylococcus Antitoxin
Staphylococcus Toxin
Tetanus Antitoxin
Tetanus Antitoxin Flocculating Serum
Tetanus Toxin
Tetanus Toxoid (Alum Precipitated)
Tetanus Toxoid (Fluid)
Thrombin
Typhoid Vaccine
Vibrion Septique Antitoxin
Vibrion Septique Toxin

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. DBS-2
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	☐	ADMINISTRATION	☐
REVIEW & APPROVAL	☐	TECHNICAL ASSISTANCE	☐
BIOLOGICS STANDARDS	☒		

12. IDENTIFY ANY COOPERATIVE UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM I)

None.

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO. (S) IF WITHIN NIH)

Does not resemble, complement or parallel research done elsewhere in the Public Health Service.

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

14. DBS-2
SERIAL NO.

15. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None.

16. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Division of Biologics Standards — INSTITUTE
2. Control Activities Branch — LABORATORY OR BRANCH
3. Control Test Section — SECTION
4. — LOCATION (IF OTHER THAN BETHESDA)
5. DBS-3 — SERIAL NO.
6. Control Tests on Biologics — PROJECT TITLE
7. Chief - Sr. Pharm. (Vacancy)
   Acting Chief - Mr. Robert W. Kolb — PRINCIPAL INVESTIGATOR(S)
8. Bacteriologist GS-12 (Vacancy) — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

The general purpose of this project is to evaluate the samples and test records of each lot of every licensed biological product which is submitted to the National Institutes of Health for release under provisions of the Biologics Control Act. The evaluation includes carrying out necessary tests, such as sterility, safety, potency and purity on samples of the biological product.

In addition to the above, this project involves: (a) research designed to improve existing in vivo and in vitro methods of testing and to develop new methods for which there is a need; (b) training activities designed to instruct and orient new government personnel and visitors from both domestic and foreign health laboratories in biologics control testing procedures as carried out at the National Institutes of Health. Cooperative studies are also made in conjunction with the World Health Organization and with other laboratories engaged in studies involving biological products.

Major findings and accomplishments.

(a) Biological products produced by laboratories holding U. S. Government license, both here and abroad, have been tested for conformance to National Institutes of Health requirements.

Project Description - Continued

(b) Complaints from the Armed Services and other users of biological products were investigated, reports and recommendations for action were made to the Chief, Control Activities Branch.

(c) Experimental studies were completed on the comparative use of the guinea pig and chick in the evaluation of Diphtheria Toxin for the Schick Test. An alternate method of assay of Schick Toxin using the chick as a test animal was proposed. The results of these studies were published.

(d) Collaborative studies were carried out with the Blood Bank Section, Laboratory of Blood and Blood Products, in designing control techniques of culturing banked human blood to detect contaminating bacterial organisms. These studies were completed and the findings published as indicated below in item 15.

(e) Other collaborative investigations have been conducted and are currently in progress with the Laboratory of Clinical Investigations, National Microbiological Institute. These investigations pertain to studies of antibody responses in leukemia and agammaglobulinemia patients following treatment and administration of various biological antigens. It is felt these studies may be beneficial in learning more about the mechanism of host resistance to infections frequently associated with the above conditions.

(f) An immunologic method for determining potency of the diphtheria component of a new fluid toxoid preparation, e.g., Tetanus and Diphtheria Toxoid Combined (For Adult Use), was developed. It is now possible to evaluate and control the potency of this product, which is used for immunization of adults who may show sensitivity to diphtheria toxoid.

(g) A suitable method for determining sterility of Poliomyelitis Vaccine has been developed.

(h) A record system was satisfactorily worked out which regulates the distribution of correspondence and protocols pertaining to blood and blood products, toxoids and multiple antigen preparations to various operators within the Division. This system enables the Chief of the Control Activities Branch to coordinate his operations between other Laboratories and Sections and thereby keep the biologics manufacturers promptly informed as to when they may expect release of their products. In addition it aids in establishing responsibility for adequate handling of the material.

Project Description - Continued

(i) Fifteen visitors representing eleven foreign countries were given orientation and training in control testing activities during 1955. The length of this training ranged from 1 day to 1 month.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. DBS-3
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	/_/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/
BIOLOGICS STANDARDS	/X/		

12. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERIVCE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM I)

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

The above project does not resemble, complement or parallel research done elsewhere in the Public Health Service.

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

14. DBS-3
SERIAL NO.

15. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

(a) Robert W. Kolb, Sara E. Branham, and Donald B. Riggs. Comparison of the guinea pig and chick in evaluation of diphtheria toxin for the Schick test. Applied Microbiology 3 (4): 242-247; July, 1955.

(b) Hugh Chaplin, Jr., Estelle Chang and Robert W. Kolb. Report of routine tests for psychrophilic and mesophilic contaminants in banked blood. Applied Microbiology 3 (4): 213-215; July, 1955.

16. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Division of Biologics Standards — INSTITUTE
2. Control Activities Branch — LABORATORY OR BRANCH
3. Pyrogens Section — SECTION
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. DBS-4 — SERIAL NO.

6. Control of the purity and safety of certain biological products as determined by the pyrogen test and the stability as determined by moisture. — PROJECT TITLE

7. Thomas F. Probey — PRINCIPAL INVESTIGATOR(S)

8. Harry W. Burruss — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

The major responsibility of this project is the testing of certain biologic products for safety as determined by the pyrogen test and for stability as measured by the moisture content of dried products. This unit is charged with the control testing of the trivalent organic arsenicals but this aspect of control testing has become a secondary activity.

Experimental studies have been concerned with the factors influencing the pyrogen test, especially the techniques of the conduct of the test, and long-term studies in experimental syphilis, particularly the development of resistant strains of T. pallidum.

This unit has been cooperating with the National Institute for Medical Research, London, England, in the development of an International Reference Preparation of Pyrogens.

Studies have been continued to determine the pyrogenicity of licensed biological products particularly with reference to the adaptability of the test as a means of determining the safety of these products for clinical administration. Studies to correlate the pyrogenicity of biologic products such as human serum albumin, as determined by results of animal tests compared with those observed following clinical administration, are especially important.

Project Description - Continued

The hemolytic factor present in fresh normal human plasma, which induces delayed thermal response in rabbits, has made it quite difficult to properly evaluate the testing of fresh plasma without inactivating this factor.

Although the available clinical experience indicates that the pyrogen test offers reliable means of eliminating, to a very large extent, products likely to induce reactions following human infusion, it is necessary that these observations be continued.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. DBS-4
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	/_/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/
BIOLOGICS STANDARDS	/X/		

12. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S) (ITEM I)

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

Does not resemble, complement or parallel research done elsewhere in the Public Health Service.

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

14. DBS-4
SERIAL NO.

15. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

16. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Division of Biologics Standards — INSTITUTE
2. Bacterial Products — LABORATORY OR BRANCH
3. Bacterial Toxins — SECTION
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. DBS-5 — SERIAL NO.
6. (a) Components of Diphtheria Toxin; (b) Evaluation of Diphtheria Toxins, Toxoids, and Antitoxins; (c) Classification of the Neisseria. — PROJECT TITLE
7. Sara E. Branham (Matthews) — PRINCIPAL INVESTIGATOR(S)
8. Marion Grabowski (GS-7) and Donald B. Riggs (GS-7) — OTHER INVESTIGATORS
9. PROJECT DESCRIPTION

a) Components of Diphtheria Toxin.

There has long been a widespread feeling that infections due to the gravis type of diphtheria bacillus (Corynebacterium diphtheriae) are more serious than infections due to other forms. It has been thought that more than one diphtheria toxin might be involved. This present study has sought to demonstrate the number of components in toxin produced by gravis and intermedius types, to study their physical and chemical properties, to separate these components, and to study their role in producing diphtheria intoxication experimentally in animals.

Preliminary agar plate precipitation tests with 32 strains of C. diphtheriae led to the choice of 3 for intensive study: 2 of gravis from fatal cases and 1 intermedius (the Park 8 strain usually employed in making toxoids and antitoxins for prophylactic and therapeutic use). Toxins were prepared with these 3 strains by growing them in a synthetic medium, filtering, dialyzing, and "freeze-drying"

Project Description (Continued)

them. These semi-purified toxins were titrated in chicks and guinea-pigs, and by flocculation. Total nitrogen determinations were made and infra-red spectroscopy gave a typical protein pattern. Samples of these toxins were studied electrophoretically both by paper strip methods and in the classical Tiselius electrophoretic apparatus, using buffer solutions of pH range 4.0 to 10.0.

By electrophoresis all toxins revealed 3 major components of different mobilities, and several minor components. The 2 gravis strains showed as many as 7 bands on paper strips.

Future work will be directed toward separating these components by continuous paper sheet electrophoresis, and in studying their properties with special reference to immunology. The fact that several outbreaks of gravis type diphtheria have occurred in the southeastern USA recently adds interest to this work.

b) Evaluation of Diphtheria Toxins, Toxoids, and Antitoxins.

The experimental work of this project has been completed, but the results have not yet been organized for publication. It was previously shown by us (published J. Immunology, 1954) that the 7-8 day chick is an excellent test animal in evaluation of diphtheria antitoxin. Since then this project was extended to include the titration of Schick test toxin (published 1955 in Applied Microbiology), and of toxoids used in immunization. The chick is dependable, readily available, and inexpensive, and is recommended for routine evaluation of these biologic products. This work will be prepared for publication in the near future.

c) Classification of the Neisseria

This taxonomic project was undertaken at the request of the Permanent Secretary of the International Committee on Bacteriological Nomenclature. During the last 28 years more than 2000 strains of the genus Neisseria, chiefly meningococci, have been studied in our laboratory and many of these (though by no means all), have been stored in a "lyophilized" state. We have been requested to check these, to choose desirable strains representing official classifications, and to send these to various "type culture collections" in various countries, including the International Catalog in Lausanne, Switzerland, which is an international clearing house. This work is being done under Dr. Branham's direction, by Mrs. Grabowski, and is well under way. All Group I meningococci (the epidemic type) have been checked to date.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. DBS-5
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	/_/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/
BIOLOGICS STANDARDS	/x/		

12. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM I)

NONE

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

NONE

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

14. DBS-5
SERIAL NO.

15. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Branham, Sara E., Kolb, Robt. W. and Riggs, Donald B. Comparison of the guinea-pig and chick in evulation of diphtheria toxin for the Schick test. Applied Microbiology, 3, 242-247, 1955.

2. Branham, Sara E. Milestones in the history of the meningococcus. Canadian Jour. of Microbiology. (In press).

3. Branham, Sara E.; Leppert, Mark; Falk, Carolyn; Alexander, Hattie and Weinstein, Louis. Bacterial meningitis. A.P.H.A., 4th Edition of Diagnostic Procedures and Reagents. (In press).

16. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Certified as Diplomate of American Board of Pathology (Specialty, Clinical Microbiology), without examination. (Branham)

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Division of Biologics Standards — INSTITUTE
2. Bacterial Products — LABORATORY OR BRANCH

3. Haemophilus Studies — SECTION
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. DBS-6 — SERIAL NO.

6. Control and improvement of pertussis vaccine and antiserum and the study of the pertussis vaccine induced mouse histamine susceptibility phenomenon. — PROJECT TITLE

7. Dr. Margaret Pittman — PRINCIPAL INVESTIGATOR(S)

8. None- need at least one more professional person — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objectives of this project are to develop ways for assuring that pertussis vaccines released for sale are adequate to afford protection to children with the minimum untoward reactions, and to find why H. pertussis causes neurological complications during the disease and sometimes following vaccination.

Methods employed: Mouse protective and toxicity tests are used to determine potency, stability and toxicity of lots of vaccines submitted for release. Cooperation in evaluation of vaccines. Various substances such as antihistamines and hormones are used to study the effect on the histamine phenomenon and the course of the infection and development of immunity.

Major findings: (1) Multiple antigens including pertussis vaccine which contain aluminum phosphate were found to be more toxic for mice than the antigens which contained other aluminum compound adjuvants. (2) The pertussis potency of vaccines submitted for release are being maintained at a satisfactory level. (3) Cooperative evaluation of a lot of vaccine proposed for the international standard pertussis vaccine was carried out.

Significance: The findings on the toxicity of antigens containing aluminum phosphate present a real problem. Work published in

Project Description - Continued

England indicates that this adjuvant is better than alum for use with diphtheria toxoid. Its use in multiple antigens containing pertussis vaccine, however, needs to be investigated.

The cooperative evaluation of vaccines on field trial from England, Scotland, Yugoslavia, Austria and the World Health Organization proposed international standards, as well as vaccines from other countries, contribute to the promotion of better pertussis vaccines world wide.

The study of the histamine phenomenon permits observations of the response of animals to a bacterium that induces an infectious disease that has many anomalous characteristics which are not understood.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. DBS-6
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	/ /	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /
BIOLOGICS STANDARDS	/X/		

12. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S) (ITEM I)

Cooperation with British Whooping Cough Committee and with Dr. Harriet Felton, University of Texas, Medical Branch, in the evaluation of field trial vaccines; and with World Health Organization in the evaluation of a lot of vaccine proposed for an international standard. No funds, facilities or personnel provided.

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

Not known.

R.P.C. 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

14. DBS-6
SERIAL NO.

15. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Stronk, M.G. and Pittman, M. The influence of pertussis vaccine on histamine sensitivity of rabbits and guinea pigs and on the blood sugar in rabbits and mice. J. Infect. Dis. 96, 152-161, 1955 (March-April)
2. Davis, D. J., Pittman, M., et. al. Susceptibility of Haemophilus aegyptius to antibiotics in embryonated eggs and in artificial media. Antibiotics and Chemotherapy 7: 363-369, 1955 (July).
3. Pittman, M. and Davis, D. J. Ineffectiveness of penicillin, rivonal lactate and tyrothricin on the development of resistance to Haemophilus aegyptius to streptomycin. Antibiotics and Chemotherapy 7: 370-374, 1955 (July).
4. Pittman, M. Symposium on Taxonomy, Part 5, The Genus Bordetella, Moreno-Lopez, 1952. Bacteriological Reviews, 19: 273, Dec. 1955.

Accepted for Publication

Pittman, M. and Alexander, Hattie. Influenza bacilli infections Chapter in Diagnostic Procedures and Reagents, 4th Ed. Am. Public Health Assoc.

Kendrick, P.L, Pittman, M. et. al. Whooping Cough Chapter in Diagnostic Procedures and Reagents, 4th Ed. Am. Pub. Health Association.

Pittman, M. Tribe Haemophileae in Bergey's Manual of Determinative Bacteriology, 7th Ed. Williams and Wilkins.

16. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

1. President, Washington Academy of Sciences - Dr. Pittman
2. Councilor, Society of American Bacteriologists, representing Washington Branch - Dr. Pittman
3. Dr. D. J. Davis received Stitt Award presented by Military Surgeons in 1955 for project on Haemophilus aegyptius. Dr. Pittman cooperated in the project but not being a member of Military Surgeons, was not eligible to share in the award.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Division of Biologics Standards — INSTITUTE
2. Lab. of Blood & Blood Products — LABORATORY OR BRANCH

3. _____ — SECTION
4. _____ — LOCATION (OTHER THAN BETHESDA)
5. DBS-7 — SERIAL NO.

6. Study of methods, preparations, equipment, testing and sterilization of blood and blood products. — PROJECT TITLE

7. J. T. Tripp, Paul Schmidt — PRINCIPAL INVESTIGATOR(S)

8. Dr. Alexander Breslow, Mr. Paul Fehnel, Miss Ann Dayton, Mrs. Eleanor Morrison, Mrs. Chappell — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives:

The main research objective is to develop methods of controlling the quality of blood and blood products and to study the problem of storage, collection, and use of blood. This includes a study of blood groups, a study of reactions caused by transfusion, a study of plastic blood bags, and a study to establish a standard period of potency for normal serum albumin, dried plasma, and blood typing serums. Important functions of this laboratory are the maintenance and operation of the Blood Bank and Transfusion Service of the Clinical Center. Major emphasis is on the control of blood and blood products processed by licensed establishments. This involves inspections of 220 establishments, testing of random samples from each establishment, and test and release of each lot of a blood product produced by each licensed manufacturer.

Methods Employed:

(1) The improvement of methods for long term storage of blood is being studied by storing red cells at -20° C. and -45° C. in the presence of glycerol-electrolyte mixtures. Viability of cells after storage is measured by both *in vitro* and *in vivo* methods.

Project Description - Continued

(2) Reactions due to transfusions are studied as they occur among patients, by immunological methods, by complete bacteriologic studies and by transfusion of packed cells, washed cells, and fresh plasma if subsequent transfusions are required.

(3) A complete panel of red cells of known antigenic makeup has been prepared and is available at all times to identify antibodies of unknown bloods. This is the basis for establishing a Blood Reference Laboratory, to start functioning July 1956. Samples of unusual bloods will be accepted from licensed establishments, thus making available to us the problem bloods from approximately two million samples tested each year by these establishments. In addition, complete genotyping of families with hereditary abnormalities will be done in a search for red cells of unusual types. Unusual cells and sera obtained in this way will be processed and used as diagnostic reagents for other tests. The unusual bloods secured in this manner will also serve as an adjunct to the low-temperature preservation of cells which can be stored for as much as six months preliminary to transfusion, and will provide a reserve of unusual bloods when needed for patients in the Clinical Center.

(4) Quality of blood is being studied by comparing different methods of blood collection. The second phase of this has been completed and is in press. Further comparisons of the quality of blood collected in other ways is in progress.

(5) Trials are being continued with a new pilot sample container for cross-match developed in this laboratory. The initial report on this study is in press. The objective is to develop a method of storing clotted blood suitable for laboratory tests when the specimen is 28 days old. This is important because of development of new techniques that will extend the dating period of blood.

(6) Evaluation blood samples are sent to licensed blood banks for testing. In this way it is possible (a) to evaluate the work performance of present licensed establishments (b) to establish criteria for the granting of new licenses (c) to further elevate the standards of blood banking and (d) to secure definitive information as to reliability of blood diagnostic reagents in the hands of users.

(7) A study is being made of methods of sterilizing biologics, including the use of ultraviolet radiation, and of filters as currently in use by the industry. Study of filtration methods is part of a long term project that is just getting underway. It will include a study of the factors involved in sterilization by Seitz type filter pads and other porous filters. The objective is to establish the fundamental factors that influence the effectiveness

of filters in an attempt to establish standards for describing filters used for specific purposes.

Major Findings:

During the past year the following findings have been made:

(1) Red cells can be stored in the frozen state for as long as 6 months and thawed with as good an in vivo survival in human volunteers as that of fresh blood.

(2) Absorption phenomena in which non-hemolytic transfusion reactions do not reoccur in a protected phase are not reduplicable in the test tube. The tolerance of even acutely ill patients for continuous, massive transfusion is very high.

(3) Serological methods have been applied to the chemical characterization of abnormal antibodies. Antibody levels have been correlated with the survival of transfused blood in auto-immune hemolytic anemia.

(4) Reports indicate that plastic containers permit the preservation of platelets and white cells. In over 100 transfusions in vivo survival of these platelets has uniformly been poor but other components of blood have been well preserved. Technical advantages of the containers are definite, and include added safety for donor and recipient.

(5) Clotted blood can be kept in special pilot sample containers for three weeks with minimal hemolysis as compared to one week for ordinary samples.

(6) Collection, packaging and shipping of blood samples in large amounts is feasible for evaluation study of blood bank functions.

(7) Albumin preparations and citric acid solutions for use with dried plasma have been stored under different conditions for periodic testing to observe changes that occur during storage. This includes the comparison of different tests now used with these preparations in an attempt to establish the usefulness of various control tests.

During the year, the blood bank provided services as follows:

(1) 2305 units of blood were collected: 1806 were given as transfusions to patients, 577 were used in the preparation of plasma, 99 were employed for laboratory research, and 134 were rejected as

unsatisfactory for administration to patients.

(2) 129 special tests were performed on blood samples from Clinical Center patients at the request of the ward physicians. Assistance in research projects of other Institutes was rendered on 104 occasions in the form of obtaining blood samples, special donor bleedings, and loans of equipment.

(3) In addition, the Blood Bank assisted in special investigations at the request of Georgetown University Hospital, Naval Medical Center, G. W. Hospital, American National Red Cross, Garfield Hospital and Army Medical Center and made the arrangements for supplying 2419 units of serum for laboratory research.

Significance of Research:

(1) Blood and blood derivatives are expensive and have a relatively short shelf-life. In the event of a national emergency a stockpile of red blood cells for transfusion would be useful and is important in the case of certain rare bloods. The work with frozen red cells has demonstrated optimum preservation of blood for up to six months.

(2) Studies on the feasibility of general use of plastic containers for blood and the development of testing methods would permit the use of light, non-breakable containers in a national emergency. This would cut down shipping costs greatly and make for safer transfusions.

(3) The identification of blood groups and antibodies will make available a large amount of material which can be used as diagnostic reagents. Human material is the only source of these reagents. Information on genetics and antigen-antibody reactions will be gained. A central service is available on a national level to identify problem bloods and coordinate findings.

(4) Evaluating the quality of blood bank laboratory work will help elevate the general national standard of medical practice and make for uniformity in procedures and will provide a basis for product requirements.

(5) Nothing is known about the changes that occur in blood products during storage. This information will permit establishment of realistic dating periods. If the stockpiled albumin and plasma is not used by the end of the dating period this study should serve as a basis for action 5 years from now.

Project Description - Continued

Proposed Course of Project:

It is proposed to continue these long term projects along present lines. Albumin stability studies will continue until 1961. The work on blood groups will be extended to include all the members of a large family group in Southern Maryland who are being studied as a cooperative NIH project. The trial and establishment of working standards for the Federal licensure of Blood Banks will continue. The Blood Reference Laboratory will make its services available to licensed blood banks throughout the nation.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. DBS-7
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	/ /	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /
BIOLOGICS STANDARDS	/X/		

12. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERIVCE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S) (ITEM I)

None

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

None

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

14. DBS-7
SERIAL NO.

15. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

16. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Division of Biologics Standards — INSTITUTE
2. Viral Products — LABORATORY OR BRANCH
3. Research — SECTION
4. — LOCATION (IF OTHER THAN BETHESDA)
5. DBS-8 — SERIAL NO.
6. Respiratory Diseases — PROJECT TITLE
7. Dr. Bernice E. Eddy — PRINCIPAL INVESTIGATOR(S)
8. W. H. Berkeley, R. Young, A. Gochenour, and W. S. Hunter (W. S. Hunter is out due to illness with tuberculosis and has been out all of 1955) — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Project: To control and improve influenza virus vaccines and pneumococcus typing serum; and to study other etiological agents responsible for respiratory disease.

Objectives: The objectives of this project are to control and improve influenza virus vaccines made with properly selected strains in order to forestall the occurrence of a devastating pandemic of influenza similar to the one which occurred in 1918-1919 and to reduce illness due to influenza in the general population; to study other etiological agents responsible for respiratory disease and to control, when necessary, pneumococcus typing serum.

Methods employed: The methods employed consist of animal inoculation, propagation in embryonated eggs and tissue cultures made from monkey renal tissue and different tissues from other animals, differential centrifugation, and serological methods.

Major findings: A potency test was worked out for a new strain of virus which was added to the influenza virus vaccines for the Armed Services in 1954-1955. Also a new standard vaccine for the mouse antigenic test for potency was chosen, evaluated and distributed to the

Project Description (Continued)

manufacturers. It must be mentioned that over 90 percent of time in the past year was spent on problems pertaining to poliomyelitis.

Significance to Biologics Standards research: The general aim of the project is to fulfill the obligation of controlling and improving influenza virus vaccines, to look ahead to the production and control of vaccines made with other etiological agents of respiratory diseases in order to have suitable tests in readiness, and to control pneumococcus typing serum.

Proposed course of project: To continue to study influenza virus strains from outbreaks or epidemics which have occurred or are occurring. Influenza virus strains which are recovered from an outbreak are usually antigenically different from the strains which are recovered from earlier outbreaks. There are two schools of thought: one, that the virulent strains which were prevalent in the past will occur again when the humoral antibodies of the population become low or lacking; the other, that influenza virus strains are constantly changing and that the old strains will not appear in later outbreaks or epidemics. Only a constant study of strains over a long period of time will answer this question.

A new strain of virus, which was added to the influenza virus vaccines for the Armed Forces last year and which will probably be used in the vaccines for 1956 and 1957, is being adapted to mice. This is a long, tedious procedure but results in a more stable and easily handled strain for production or test purposes.

The effect of radiation for possibly potentiating the virulence of influenza virus in animals is being investigated jointly with a group in another Institute (NIAMD). With radiation a possible threat to large numbers of people, it is important to know what might be expected from latent viruses or viruses of low virulence. In part, this study can be worked out while adapting a new strain to mice.

The APC or ARD viruses will be studied in tissue culture and by other means with a view to establishing means of control in the event that vaccines made from these viruses become practicable.

Pneumococcus typing serum will be controlled when submitted. If time permits, comparisons will be made of some of the American and Danish types.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. DBS-8
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	/_/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/
BIOLOGICS STANDARDS	/X/		

12. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM I)

NONE

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

The project complements some experimental work on vaccines in humans in NMI and on vaccine studies by the Armed Forces Commission on Influenza. It is not the same.

A group in NIAMD is cooperating by exposing animals to known doses of radiation. This is a small part of the activities of the project.

R. P. C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

14. DBS-8
SERIAL NO.

15. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

NONE

16. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Honorary degree - Doctor of Science at Marietta College, June 1955.

Relating to poliomyelitis - An invitation to a WHO meeting on poliomyelitis in Stockholm, Sweden, November 21-25, 1955.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities
Project Description Sheet

1. Division of Biologics Standards — INSTITUTE
2. Laboratory of Viral Products — LABORATORY OR BRANCH

3. Testing — SECTION
4. _____ — LOCATION (IF OTHER THAN BETHESDA)
5. DBS-9 — SERIAL NO.

6. Poliomyelitis Vaccine Testing
PROJECT TITLE

7. George A. Hottle, Ph.D.
PRINCIPAL INVESTIGATOR(S)

8. Drs. Abinanti, Baron, Crawford, Eddy, Li, and McKinstry
OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Objectives: The objectives of this project are to investigate methods for the safety and potency testing of poliomyelitis vaccine.

Methods: For safety testing the vaccine is concentrated by centrifugation or chemical precipitation and inoculated either in the concentrated or unconcentrated form into monkey kidney cell culture or other tissue culture and into monkeys by the intracerebral, intraspinal and intramuscular routes. The tissue cultures and the monkeys are observed for signs of infection with poliomyelitis virus.

For potency testing the vaccine is injected into animals of various species and the development of antibodies is studied.

Major Findings: The addition of 1% calf serum to vaccine to which about 10 tissue culture infecting doses of virus were added brought about the sedimentation of at least 90% of the virus by a 4-hour centrifugation run at 30,000 RPM in the Spinco Centrifuge.

Significance: In order to understand the nature of any live virus which may persist in poliomyelitis vaccine during the inactivation process, it is necessary to know how much virus is present at any stage. When dealing with minimal amounts of virus the question of either testing large volumes of unconcentrated vaccines or small volumes of concentrated vaccines becomes important. By the study of concentration methods the presence of possible masking or interfering substances can be approached. The development of methods for

Project Description - Continued

detecting poliomyelitis virus is the purpose of this project.

Proposed course of project: The general approach is to apply techniques for virus detection to vaccines containing small amounts of native (added) virus and to vaccine containing small amounts of residual (formalin-treated) virus. Concentration by centrifugation, zinc precipitation, and ultrafiltration will be studied with the vaccines referred to above.

The study of antibody formation in chickens and in rabbits is being planned in order to compare the antigenicity of vaccines under production by the vaccine manufacturers.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. PBS-9
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	/_/	ADMINISTRATION	/_/
REVIEW & APPROVAL	/_/	TECHNICAL ASSISTANCE	/_/
BIOLOGICS STANDARDS	/X/		

12. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO.(S) (ITEM I)

Cutter Laboratories
Berkeley, California

Eli Lilly and Company
Indianapolis, Indiana

Parke, Davis and Company
Detroit, Michigan

Pitman-Moore Company
Indianapolis, Indiana

Sharp and Dohme
Glenolden, Pennsylvania

Wyeth Laboratories, Inc.
Marietta, Pennsylvania

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

Does not resemble, complement or parallel research done elsewhere in the Public Health Service.

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

14. DBS-9
SERIAL NO.

15. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

16. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Division of Biologics Standards — INSTITUTE
2. Laboratory of Viral Products — LABORATORY OR BRANCH

3. Hepatitis Study — SECTION
4. _____ — LOCATION (IF OTHER THAN BETHESDA)

5. DBS-10 — SERIAL NO.

6. Study of serum hepatitis and infectious hepatitis — PROJECT TITLE

7. Dr. Roderick Murray — PRINCIPAL INVESTIGATOR(S)

8. None — OTHER INVESTIGATORS

9. PROJECT DESCRIPTION

Studies on serum hepatitis and infectious hepatitis.

Objectives: The main objective of this project has been to study methods of sterilizing blood and blood derivatives with respect to the agent(s) of viral hepatitis. Secondary objectives are the study of hepatic dysfunction in individuals who have had hepatitis (follow-up study), and to attempt to infect Rhesus monkeys with materials known to contain the agent(s) of viral hepatitis.

Methods Employed: Methods of sterilizing blood and blood derivatives have been investigated by means of studies conducted with the cooperation of prisoner volunteers in Federal prisons. This work came to a close in 1954.

Evidences of residual hepatic dysfunction are being sought in clinical and laboratory follow-up studies of individuals who developed hepatitis while acting as

Project Description - Continued

volunteers. These persons are contacted either in prison or after return to civil life, as opportunity arises, in periods of time up to three years. It is believed that valuable information on the occurrence and severity of residual sequellae of hepatitis will be obtained in the course of this study, particularly as baseline "normal" data are available for each individual for a period of approximately three months just prior to the onset of hepatitis.

A program of study of 90 Rhesus monkeys inoculated with known icterogenic material and followed by liver function tests was concluded during the past year.

Major Findings: It is apparent that a considerable proportion of those who had hepatitis show minor disturbances in liver function tests. The proportion is greater among those who had hepatitis without icterus than among those who had hepatitis with icterus. None of these abnormalities in liver function tests appear to be of clinical significance however. Despite suggestive changes in liver function tests, particularly in the cephalin flocculation test, there was no evidence that the agent of homologous serum hepatitis could be transmitted to Rhesus monkeys.

Significance: Persons who receive blood transfusions or administrations of blood derivatives such as plasma run the hazard, sometimes mortal, of infection with the agent of serum hepatitis. The acquisition of information concerning the transmission of serum hepatitis is difficult and uncertain because of the absence of *in vitro* and *in vivo* tests for the agent(s) involved. Transmission of the infection to any animal other than man has not been accomplished. The series of studies, conducted as part of the present project, and during the course of which much new information concerning the resistance of the agent(s) to physical (heat, ultraviolet irradiation) and chemical (Beta-Propiolactone, sulfur mustard, fractionation) processes was obtained, were carried out through the cooperation of human volunteers.

Project Description - Continued

Proposed course of Project: Follow-up studies of volunteers will be pursued during the ensuing year, and with the increased tissue culture and other facilities for studying viruses at the DBS, it is hoped that it will be possible to subject some of the serums and other materials in storage to such study, in the hope of propagating the virus.

Note: During the present year, this work has been seriously dislocated by the severe calls on the time of the senior investigator made by problems arising from the poliomyelitis vaccine situation which followed April 12, 1955.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. DBS-10
SERIAL NO.

11. BUDGET ACTIVITY:

RESEARCH	/ /	ADMINISTRATION	/ /
REVIEW & APPROVAL	/ /	TECHNICAL ASSISTANCE	/ /
BIOLOGICS STANDARDS	/X/		

12. IDENTIFY ANY COOPERATING UNITS OF THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 OR 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S) (ITEM I)

A. Armed Forces Epidemiological Board provided funds.

B. Bureau of Prisons, Department of Justice, has provided hospital facilities during 1954.

13. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH: (BY SERIAL NO.(S) IF WITHIN NIH)

Does not resemble, complement or parallel research done elsewhere in the Public Health Service.

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

14. DBS-10
SERIAL NO.

15. LIST PUBLICATIONS OTHER THAN ABSTRACTS FROM THIS PROJECT DURING CALENDAR YEAR 1955:

1. Effect of Ultraviolet Radiation on the Infectivity of Icterogenic Plasma. Roderick Murray, J. W. Oliphant, J. T. Tripp, Bettylee Hampil, Frank Ratner, William C. L. Diefenback and Herman Geller. J.A.M.A., 157:8-14, Jan. 1, 1955.

2. The Problem of Reducing the Danger of Serum Hepatitis from Blood and Blood Products. Roderick Murray, Wm. C. L. Diefenbach, Herman Geller, N. C. Leone, Frank Ratner. N. Y. State Journal of Medicine, 55(8):1145-1150, April 15, 1955.

3. Viral Hepatitis. Roderick Murray. Bull. of the N. Y. Academy of Medicine, 31(5):341-358, May, 1955.

16. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

Named member of Commission on Viral Infections, Armed Forces Epidemiological Board.

1. Office of Director 2. Biometrics Branch

3. 4. 5.

6. Mortality Among Workers in Cigarette Factories

7. Harold F. Dorn
William S. Baum, NCI

8.

9. Objectives: Ascertain whether mortality from specific causes--respiratory cancer, coronary heart disease, etc.--is higher among cigarette factory workers than for the general population.

Methods Employed: Records were assembled of deaths among employees of the American Tobacco Company from October 1946 through December 1952.

Major Findings: The death rate from all causes except coronary heart disease and respiratory cancer was lower among the cigarette factory workers than the corresponding rate for the total population of Virginia and North Carolina where most of the employees worked. No difference was found in the mortality rates for coronary heart disease and respiratory cancer in the two population groups.

Significance: This study did not support the statement that cigarette factory workers are subject to special health hazards.

Proposed Course of Project: This project has been completed.

10.

11.

12.

13. Field Investigations and Demonstration Branch, NCI

14.

15.

16. Harold F. Dorn and William S. Baum, "Mortality Among Workers in Cigarette Factories," Industrial Medicine and Surgery 24, 239-241, 1955.

17. None

1. Office of Director 2. Biometrics Branch

3. 4. 5.

6. Variation in Mortality Among Persons Living in Metropolitan and Non-metropolitan Areas.

7. Harold F. Dorn

8.

9. Objectives: Determine differences in cause of death of persons living in (a) the central county of each metropolitan area in the United States, (b) the adjacent metropolitan counties, and (c) non-metropolitan counties; study variation in mortality by economic regions of the United States.

Methods Employed: Retabulation of all death records for 1949-1951 in the United States. The entire United States has been subdivided into 119 economic regions which contain 168 metropolitan areas. Data for about 90 causes of death will be tabulated for each economic region sub-classified into the three county groups listed above.

Findings: The punched-cards are now being prepared preparatory to making the tabulations.

Significance: This will be the first comprehensive analysis of differences in cause of death of persons living in different types of metropolitan and non-metropolitan areas in the United States. It has been designed to determine whether any general unhealthy aspects of living in large, densely population metropolitan areas can be detected.

Course of project: The basic tabulations should be completed during the summer of 1956.

10.

11.

12.

13. The Division of Special Health Services, Community Air Pollution Medical Program is paying for the preparation of the tabulations.

14. This project is one of a series of studies of the effect of air pollution upon health sponsored by the Division of Special Health Services.

15.

16. None

17. None

1. Office of Director 2. Biometrics Branch

3. 4. 5.

6. Relationship of Use of Tobacco to Health

7. Harold F. Dorn
William S. Baum, NCI

8.

9. Objectives: To study the causes of death of males who have used tobacco in comparison with those who have not used tobacco.

Methods Employed: A questionnaire requesting information concerning the use of tobacco was sent to about 300,000 veterans who had U. S. Government Life Insurance policies. After each policyholder dies, medical data concerning the cause of death are obtained from the hospital where the death occurred or from the attending physician. Mortality rates for specific causes of death will be computed for policyholders classified by history of use of tobacco, age, occupation, and residence.

Findings: Data still are being collected.

Significance: This study will provide data on the relative effect of the three major environmental factors--use of tobacco, occupational hazards, and residence--thought to be associated with the rapid increase in lung cancer.

Course of Project: The project will be continued for a number of years until the number of deaths is sufficient to carry out the analysis planned.

10.

11.

12.

13. Field Investigations and Demonstrations Branch, NCI

14.

15.

16. None

17. None

1. Office of Director 2. Biometrics Branch

3. 4. 5.

6. Mortality by Occupation and Socio-Economic Groups in the United States.

7. Harold F. Dorn
Iwao M Moriyama (NOVS)
Lillian Guralnick (NOVS)

8.

9. Objectives: Provide data on variations in causes of death of males who have worked in specific occupations; study the relationship of general socio-economic status to mortality.

Methods Employed: The sources of data are death certificates for males 20-64 years of age who died during 1950. The basic population statistics are from the 1950 Census of Population.

Findings: During the past year, the coding and card punching were completed. Tabulation plans were prepared and preliminary tables were run. Plans for the final tables to be published are now being prepared.

Significance: This project is the first comprehensive study of occupational mortality in the United States. It should provide data showing the occupation with a higher than average mortality from specific diseases.

Course of project: During the coming year, final tabulations will be completed and the analysis of the data will be started.

10.

11.

12.

13. National Office of Vital Statistics

14.

15.

16. None

17. None

1. OFFICE OF THE CHIEF 2. Laboratory Aids Branch

3. Animal Production

6. Studies on strain differences in biological responses in the small laboratory mammals produced by the Animal Production Section.

7. Dr. George Jay

Objectives: There are two main objectives to this project; (1) to study the genetic aspects of strain differences, and (2) to enhance the experimental value of the strains in production by providing a more complete biological characterization of each strain.

Methods: By using various experimental procedures (biochemical, physiological, pharmacological, etc.), strain differences in response to drugs, tissue transplants, infectious organisms, diets, etc. have been and will continue to be studied, either within the Animal Production Section proper, or in cooperation with the various NIH research areas.

Major Accomplishments: The major accomplishments during the past year have been (1) the demonstration of strain differences in mice in response to the barbiturate drug, Hexobarbital (Evipal), and (2) the demonstration of strain differences in mice in the incidence of spontaneous osteoarthritis. This last accomplishment was in cooperation with NIAMD.

Significance to NIH Research: A strain difference in the manifestation of a biological response has a genetic significance, for if such a difference is real and consistent, there is probably a genetic basis for it. Thus, the use of such a difference provides an experimental approach for the research worker for controlled studies on the mechanisms of various biological processes. Furthermore, the identification of these strain differences adds to the general biological characterization of these strains, which enhances their value as research materials.

Proposed Course of this Project: The next year will be devoted to further studies of this nature, and in particular, studies on osteoarthritis in mice, dietary liver necrosis in various rat strains, and enzyme studies in mice with respect to drug metabolism. These three studies will be made in cooperation with research personnel in NIAMD, and NIMH. In addition, a study on strain differences in mice in response to other barbiturate drugs will be done in the Animal Production Section proper.

1. Office of the Chief 2. Laboratory Aids Branch

3. Animal Production

6. Research and development of equipment for housing, feeding, watering and handling laboratory mammals.

7. Mr. Samuel Poiley

Objectives: The objective of this project is the design and development of new caging equipment, revisions of existing cages as required for specific uses, and the design and development of handling devices for laboratory mammals that will be applicable to the requirements of the production colonies and/or research projects.

Methods Employed: Drawings and specifications are made, and prototypes are constructed and used under actual conditions in both production and experimental animal rooms.

Major Accomplishments: The major accomplishments during the past year have been the redesigning of monkey and chimpanzee cages; the development of a disposable cage pan for general purpose and monkey cages; the adapting of conventional watering and feeding devices to monkey cages; the development of handling equipment for monkeys: the development of a fibre glass monkey cage. In addition to these, other accomplishments have been concerned with revisions in metabolic cages of various types, advising both Governmental and non-Governmental Institutions in matters of equipment design, construction, and purchase, and advising NIH research staff on the uses of caging equipment.

Significance to NIH Research: The design and construction of caging equipment and accessories currently in use was based on past experiences. Present experiences indicate that new designs and construction are needed in some types of equipment to further satisfy the biological requirements of laboratory animals not fully accommodated by the present designs, and also because of changing trends in research. The new designs will promote the well-being of animals, and will, perhaps, aid the quality and quantity of animals produced.

Proposed Course of the Project: The present course will be followed, in that new designing and developing will continue as long as the need for such equipment is apparent. At the present time a cage design is being worked out that will be adaptable for various small mammals, and that can be accommodated on stands already available. In addition to this effort, trials are underway on magnetic cage door fasteners, disposable transporting boxes, revisions of cat cages so they can be used for guinea pigs or rabbits, and other ideas of a similar nature that will aid in increasing the efficiency of animal production and maintenance.

1. OFFICE OF THE CHIEF 2. Laboratory Aids Branch

3. Animal Production

6. Animal husbandry practices and procedures for the efficient production and maintenance of laboratory mammals.

7. Mr. Samuel Poiley

Objectives: The objective of this project is to revise old husbandry practices and procedures, and to devise new ones applicable to large production colonies, that will promote the quality and quantity of animals produced.

Methods Employed: Experimental tests are designed and employed to measure the efficacy of existing husbandry practices and procedures where there is reason to question the use of such procedures. Such tests make use of growth rates, weaned litter size, total numbers produced, incidence of spontaneous and chronic diseases, incidence of pregnancies, etc. Any new practices and procedures are tested by these criteria, and are either adopted or discarded. In general husbandry practices and procedures encompass feeding, housing, cleaning, breeding, weaning, the use of any therapeutic devices such as antibiotics, sulfa drugs, etc.

Major Accomplishments: During this past year disease-free colonies of non-inbred guinea pigs have been successfully established on a production basis. This has been largely due to the rigid husbandry practices and procedures established. Similarly, the rabbit colony has been freed of Coccidiosis and Salmonellosis, and respiratory infections have been minimized. Considerable progress has been made by the use of new and revised procedures in minimizing respiratory infections in the rat colonies. Some progress has been made in controlling spontaneous infantile diarrhea in mice, and new husbandry practices were instituted in the monkey quarantine colony.

Significance to NIH Research: Since there have been marked improvements made in the animal colonies during the past year, all of the revisions of old practices and the adoption of new ones have been of considerable value in improving the quality of the animals produced. The significance of this with respect to the over-all research program of NIH is obvious.

Proposed Course of this Project: The present course will continue. As long as there are things to be learned in the production, care, and maintenance of small laboratory mammals, there will always be a need for a project of this sort. Efforts will continue in the rat colony to further minimize the respiratory infection problem, in the mice to control infectious infantile diarrhea, in the rabbits to eliminate respiratory infections, and on any related problems that may arise during the year. In addition, a breeding program in an effort to develop a strain of rats resistant to respiratory infection is underway and will be continued during the next year.

1. OFFICE OF THE CHIEF 2. Laboratory Aids Branch

3. Animal Hospital Section

6. Orthopedic Surgery in the Dog

7. Dr. William Gay

Objectives: (1) To evaluate the use of metals as replacements for long bone shafts.

(2) To evaluate the use of titanium as a material for bone prostheses.

Methods Employed: Surgical removal of a segment of bone shaft and replacement with metallic prosthetic pieces.

Major Findings: In nine operative procedures, bone overgrowth on the metallic prostheses has occurred and six of these animals have recovered to the extent of normal ambulation within one year following surgery. Titanium has proven, to date, to be a valuable light-weight, non-irritating metal for use as a prosthetic device in bone shaft replacement.

Significance to Research: Titanium has not been used in human medicine for bone prostheses. Due to its light weight (50% that of stainless steel), and its non-irritating properties, these experiments would indicate that this metal has possibilities for human surgery procedures.

Proposed Course of Project: Continuation of procedures and long-term follow-up on present cases.

1. OFFICE OF THE CHIEF 2. Laboratory Aids Branch

3. Instrument Section

6. Instrument Development - Clinical & Research

7. Mr. Laurence Crisp

Objectives: To provide instrumentation to further advance the medical and research arts (endeavor).

Methods Employed:

(a) Observation of instrument needs.

(b) Creation of ideas.

(c) Engineering planning of instrument.

(d) Prototype development & construction.

(e) Test & evaluation by clinical and research investigators.

Major Findings:

(1) No independent research projects have been conducted since January 1, 1955, as no provisions were made under fee-for-service to conduct studies. Prior to the above date, the Instrument Section conducted research and developed working models of:

(a) Peristaltic pump.

(b) Continuous flow perfusion pump.

(c) Fraction collector.

(d) Droplet Counters.

(e) Fluid control apparatus.

(f) Pipette washing machinery.

(g) Tooth sectioning & polishing machine.

Proposed Course of Project: Until such time as funds are made available, no independent projects can be undertaken by this section.

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Division of Research Services
INSTITUTE OR OTHER NIH UNIT

2. Sanitary Engineering Branch
LABORATORY, BRANCH OR DEPARTMENT

3.
SECTION

4.
LOCATION (IF OTHER THAN BETH.)

5. 12170 -1
SERIAL NO.

6. Laboratory and Hospital Sanitation Management
PROJECT OR ACTIVITY TITLE

7. Chief, Sanitary Engineering Branch - Donald L. Snow
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. Senior Sanitary Engineer - Floyd B. Taylor
Chief, Insect and Rodent Control Section - Robert D. Murrill
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Objectives:

The objective of this program is to investigate, establish and apply standards of sanitation for laboratories and hospitals through existing and new patterns of administrative relations.

Methods employed:

Methods employed include utilization of the laboratory and hospital sanitary surveys - including the securing of independent, substantiating laboratory data, design of administrative devices such as collective committee action and developing supervisor and employee training aids.

The existence of the extensive laboratory and patient-care facilities on the NIH grounds makes immediately available a considerable source of practical experience and information at minimum expense.

Major findings:

Major findings to date point to the practicality and economies, within limitations, of centralizing certain laboratory ancillary services, such as glassware cleaning, waste disposal, refuse can cleaning, and cleaning of animal cages and their appurtenances.

Project Description Sheet

Significance to research:

The significance to the conduct of research in the medical and biological sciences, both within and without the NIH, is immediate and direct as evidenced by (a) the growing demand for such services at the NIH and (b) the intense interest displayed by scientists and research administrators outside the NIH.

Proposed course of project:

The proposed course of action in the project includes particular emphasis during the present and following fiscal years towards (a) completion of environmental health surveys of all major research buildings on the NIH grounds; (b) development of standards for animal room sanitation; and institution of animal room attendants' training courses; (c) technical studies and development of facilities for disposal of laboratory chemical wastes.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12170
SERIAL NO.

11. BUDGET DATA:

12. BUDGET ACTIVITY:

RESEARCH (SERVICE) [ ] ADMINISTRATION [X]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [X]

13. IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Not Applicable.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not Applicable

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. 12170
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

"In Building or Remodeling--Don't Neglect Waste Disposal Planning," Report of A.P.H.A. Committee on Hospital Facilities (D. L. Snow, Chairman), HOSPITALS, Vol. 30, No. 3, February 1956.

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Division of Research Services — INSTITUTE OR OTHER NIH UNIT
2. Sanitary Engineering Branch — LABORATORY, BRANCH OR DEPARTMENT
3. _____ — SECTION
4. _____ — LOCATION (IF OTHER THAN BETH.)
5. 12170-2 — SERIAL NO.

6. Distilled Water Production
PROJECT OR ACTIVITY TITLE

7. Principal Investigator - Harry Stierli, Senior Sanitary Engineer
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. Donald L. Snow, Chief, Sanitary Engineering Branch
John L. S. Hickey, S.A. Sanitary Engineer (R)
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Objectives:

Determine the criteria for distilled water used in biological research. Investigate types and materials of construction for stills, storage tanks, and distribution piping to determine their effect on distillate quality. Study pretreatment of water supplied to stills. Compare economics of production of distilled water with quality of the distillate.

Methods employed:

Criteria for distilled water for medical and biological research problems are determined from required precision of experiments. Distillate from various types of stills, tanks, and distribution piping systems installed at the NIH are checked for conductivity and presence of trace metals, such as copper. Performance of special equipment will be studied for production of pyrogen-free water of low conductivity and freedom from trace element contaminants. Effect of pre-treatment, such as demineralization of water supplied to a still, will be observed. Initial cost, operation costs and maintenance costs will be compared for several distilled water systems.

Major findings:

Many research activities require a uniformly high quality of distilled water for satisfactory investigational work. It has been established that studies on enzyme systems, proteins, etc. necessitate use of water free of trace element contaminants. Work is needed in controlling the

quality of the distilled water to insure that it will meet these requirements.

Significance to NIH research:

Improvement of quality and uniformity of quality of distilled water produced at the NIH will directly benefit the conduct of much research at the NIH.

Proposed course of project:

A portion of the next calendar year will be devoted to studying the performance of special equipment for production of distilled water for laboratory use. The study will include tests on the effect of pretreatment of water supplied to a still. It will also include a comparison of costs of production of distilled water with quality of distillate.

R.F.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12170
SERIAL NO.

11. BUDGET DATA:

12. BUDGET ACTIVITY:

RESEARCH (SERVICE) [ ] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [X]

13. IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Not Applicable.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not Applicable.

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. 12170
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Division of Research Services — INSTITUTE OR OTHER NIH UNIT
2. Sanitary Engineering Branch — LABORATORY, BRANCH OR DEPARTMENT
3. _____ — SECTION
4. _____ — LOCATION (IF OTHER THAN BETH.)
5. 12170 - 3 — SERIAL NO.

6. Laboratory Glassware Surface Contaminants and Their Removal

7. Principal Investigator - Harry Stierli, Senior Sanitary Engineer
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. Donald L. Snow, Chief, Sanitary Engineering Branch
John L. S. Hickey, S.A. Sanitary Engineer (R)
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Objectives:

Determine the criteria for clean laboratory glassware used in research at the NIH. Study methods of removal of soils on glassware surfaces, including trace toxic elements. Improve detergents used for washing of laboratory glassware. Establish effective quality controls for central cleaning facilities.

Methods employed:

The visual inspection of surfaces combined with physical, chemical, and bacteriological tests and statistical analysis are used to determine cleanliness of laboratory glassware. Various machine methods and detergents are tested using a standard soil. Chemical, physical and performance tests are conducted on selected and newly developed detergents. Methods for inspection and testing laboratory glassware surfaces for trace contaminants will be studied.

Major findings:

Common forms of laboratory glassware can be satisfactorily cleaned by machine methods in a central facility. Glassware used in tissue culture studies requires special attention and further study. A detergent for mechanical washing of laboratory glassware has been developed for NIH use.

R.P.C. - 1 (Concl'd) Analysis of NIH Program Activities
December 1955

Project Description Sheet

Significance to NIH Research:

Uniformly clean laboratory glassware is basic to the conduct of medical and biological research.

Proposed course of project:

A continued study of the criteria for clean laboratory glassware will be conducted. Additional studies will be made on methods of removal of soils from glassware surfaces. Particular attention will be given to the development of effective quality controls for cleaned glassware.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12170
SERIAL NO.

11. BUDGET DATA:

12. BUDGET ACTIVITY:

RESEARCH (SERVICE) [ ] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [X]

13. IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

Not Applicable.

14. IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

Not Applicable.

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. 12170
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None

R.P.C. - 1
December 1955

Analysis of NIH Program Activities

Project Description Sheet

1. Division of Research Services — INSTITUTE OR OTHER NIH UNIT
2. Sanitary Engineering Branch — LABORATORY, BRANCH OR DEPARTMENT
3. _____ — SECTION
4. _____ — LOCATION (IF OTHER THAN BETH.)
5. 12170-4 — SERIAL NO.

6. Effective Sealing of Conductive Terrazzo Operating Room Flooring
PROJECT OR ACTIVITY TITLE

7. Principal Investicator, Harry Stierli, Senior Sanitary Engineer
PRINCIPAL INVESTIGATOR OR OTHER RESPONSIBLE HEAD OF ACTIVITY

8. John L. S. Hickey, S. A. Sanitary Engineer (R)
OTHER SENIOR INVESTIGATORS OR PRINCIPAL ASSISTANTS

9. PROJECT DESCRIPTION

Objectives:

1. To find an effective floor sealer suitable for conductive terrazzo floors.

2. To develop a quick, efficient method of applying the sealer to the floors so as to make the floors impervious to moisture without impairing the conductive properties of the flooring.

Methods employed:

Different sealers are applied to sections of conductive terrazzo floors in varying amounts using several methods of application. The sections are afterward tested for imperviousness and for any change in conductive properties. An operating room in the Clinical Center Surgery Area is being used for the tests.

Major findings and problems:

Two sealers have been found to be suitable. However, extreme care must be used in the application of sealers in order not to insulate the flooring. Using the natural fluorescing properties of these sealers, it has been found that the uniformity of their application can be effectively controlled with UV light.

R.P.C. - 1 (Concl'd) Analysis of NIH Program Activities
December 1955

Project Description Sheet

Proposed course of project:

It is planned to adapt the experimental methods to mass application of sealer to conductive terrazzo floors. Sealing of all conductive terrazzo flooring at NIH, and publication in detail of methods used (especially control methods) is also planned. Periodic tests will be conducted afterward to determine how the sealer holds up under continued use.

R.P.C. - 2
December 1955

Analysis of NIH Program Activities

Budget Data Sheet

10. 12170
SERIAL NO.

11. _____
BUDGET DATA:

12. _____
BUDGET ACTIVITY:

RESEARCH (SERVICE) [ ] ADMINISTRATION [ ]

REVIEW & APPROVAL [ ] TECHNICAL ASSISTANCE [X]

13. Oral consultation with Division of Hospital Facilities, DHEW
IDENTIFY ANY COOPERATING UNITS OF NIH, THE PUBLIC HEALTH SERVICE, OR OTHER ORGANIZATIONS, PROVIDING FUNDS, FACILITIES, OR PERSONNEL FOR THIS PROJECT IN EITHER 1956 or 1957: IF COOPERATING UNIT IS WITHIN NIH INDICATE SERIAL NO(S):

None

14. Sealers for Conductive Terrazzo Floors in Hospitals: Department of Health Education and Welfare, Division of Hospital Facilities, January 1954.
IF THIS PROJECT RESEMBLES, COMPLEMENTS, OR PARALLELS RESEARCH DONE ELSEWHERE IN THE PUBLIC HEALTH SERVICE (WITHOUT INTERCHANGE OF PERSONNEL, FACILITIES OR FUNDS), IDENTIFY SUCH RESEARCH:

R.P.C. - 3
December 1955

Analysis of NIH Program Activities

Honors, Awards, and Publications Sheet

15. 12170
SERIAL NO.

16. LIST PUBLICATIONS RESULTING FROM THIS PROJECT DURING CALENDAR YEAR 1955:

None

17. LIST HONORS AND AWARDS TO PERSONNEL RELATING TO THIS PROJECT DURING CALENDAR YEAR 1955:

None