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PROGRESS REPORT

Work performed under AEC Contract No. AT-(40-1)-288 - Title I.
The Bowman Gray School of Medicine of Wake Forest College.

Distribution and turnover of sodium and potassium in acute infections.

Dr. George T. Harrell, Jr., Project leader July 1, 1950 to March 15, 1951

Facilities: The laboratory as previously constructed has proved in use to be very satisfactory. No changes have been made in the design or construction of the laboratory. The major items of equipment previously purchased have proved quite satisfactory. No different types of equipment have been needed this year. Additional counters, shields, and other items for expansion of the existing equipment have been purchased.

Personnel: Dr. Jerry K. Aikawa completed his AEC Fellowship. We have been able to obtain for him a fellowship from the American Heart Association which will enable him to continue work with us for at least another year. Financial support for him may be required by 1952.

Miss Eloise Rhoades, technician, has now been completely trained in our techniques and is proving quite satisfactory.

Claude Taylor, the negro technician, is proving satisfactory with the animal experiments.

Dr. Ernest Yount continues to assist in the design of experiments affecting human beings. He also is directing the experiments with use of iodine-131 which are not directly related to the primary research project.

Early in the year we were able to utilize house officers in the performance of certain groups of experiments. The majority of our house staff has now been called to military service and the shortage of personnel at this level is critical. Our human work may suffer during the next few years unless personnel not eligible for military service can be found.

Isotopes: Considerable time is still lost due to the vagaries of air transport. We have attempted to re-design experiments so that sodium-24 can be used even if it is late in arriving. In the case of potassium-42, however, it has proved impossible to design experiments which would give valid results should the isotope be late in arriving. The specific activity of the material received from Oak Ridge is such that if the shipment is as little as 12 hours late it would require the administration of a chemically toxic dose in order to achieve a satisfactory counting rate. This difficulty has made it impossible to use three of the first four shipments of potassium which started February 5. We have tried surface transportation by truck and air transportation by several different routes with about equal lack of success.

Animals: The animal breeding program mentioned in the previous progress report has been started. Very few animals were raised during the winter, but a few animals are now becoming available. It is not yet possible to estimate whether we can raise all the animals we need or not.

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Results of Experiments

Concept of Membrane Permeability Defect

A discussion of the concept of alterations in membrane permeability with various infectious and immune processes was presented at the June, 1950, meeting of the American Medical Association in the Section on Experimental Medicine and Therapeutics. This report is to be published in the JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION. In it selected examples of our data on fluid volume changes in Rocky Mountain spotted fever, trichinosis, serum sickness and drug reactions in human beings are presented. The effect on intracellular fluid of heavy metal poisoning of enzyme systems by arsenic and the reversal after administration of BAL gives a clue as to possible mechanisms in infection.

Studies in Immunophysiology

As an extension of the original work on induced hypersensitivity in animals, the conditions of the experiments were slightly modified in order to decrease random variations. The physiologic effects of the injection of human plasma into rabbits were correlated with immunologic changes. Evidence for alterations in capillary and cell membrane permeability were found, which were thought to be related to the humoral antibody response. This work entitled, "The Immunophysiology of Serum Sickness: Alterations in the Blood Volume and Thiocyanate Space in Relation to the Development of Humoral Antibodies in the Rabbit," J. K. Aikawa and G.T. Harrell, has been accepted for publication in the Journal of Clinical Investigation and is scheduled for the April, 1951, issue.

A similar study was performed with purified human plasma fractions, specifically, albumin and globulin. Although the results varied quantitatively, the changes were qualitatively similar to those observed in the studies with human plasma. Here again, it was thought possible to attribute changes in blood volume, thiocyanate space and hematocrit as due to defects in membrane permeability as a result of immunologic phenomena. This work has been summarized and has been submitted for publication.

The current opinion is that *in vivo* antigen-antibody reactions are mediated by certain substances resident in the body in an inactive state, and which are released during the antigen-antibody reaction and thus cause the systemic effects. Histamine has often been implicated. It also is not established whether the increase in capillary permeability which is known to occur with various hypersensitive states, is due to damage to the endothelial cells or to an alteration in the intercellular cement substance. Hyaluronic acid is a constituent of the intercellular cement substance and is depolymerized by the enzyme hyaluronidase. In an attempt to determine whether these two substances contribute to the biochemical defect in hypersensitivity reactions, normal rabbits were injected with large doses of histamine and hyaluronidase. It was found that neither the plasma volume nor the thiocyanate space was altered in either group of animals. It therefore was concluded that neither of these substances was solely responsible for the changes previously observed in *in vivo* antigen-antibody reactions. This study is in the final manuscript stage.

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Preliminary studies with the passive transfer method are still in progress in order to determine the optimum dose of antigen and antibody required consistently to reproduce serum sickness and certain physiologic changes in rabbits. The difficulty to date has been in obtaining sufficiently high-titered sera for injection. After repeated sensitization of several animals, it now appears that such sera can be produced.

Comparative Studies with Thiocyanate and Radiosodium

In the previous progress report, it was mentioned briefly that a comparative study of the thiocyanate and radiosodium methods as indices of the volume of the extracellular space was in progress. These determinations were made in individuals with various pathologic physiologic states. To date, no data are available in the literature on the reliability of these two methods under abnormal conditions, although a report of a limited study in normal individuals is available. To date, we have performed studies with radiosodium-24 in more than 50 individuals and compared them with values for thiocyanate done simultaneously. In the group as a whole the two spaces checked as closely as in normal individuals. In specific diseases, however, wide divergence between the two methods was encountered which was thought to reflect differences in the pathologic-physiologic responses between different diseases. Much more work needs to be done before any definitive conclusions can be drawn.

A brief preliminary report of this work was presented at the November, 1950, meeting of the Southern Medical Association, in the Section on Medicine. This report is to be published soon in the SOUTHERN MEDICAL JOURNAL.

A discussion of the applicability of such methods in medical research and a demonstration of the actual methods used in this laboratory for such studies was given at the Oak Ridge Institute of Nuclear Studies at the Advanced Training Course in February, 1951.

A comprehensive analysis of the results is planned in the near future, after more data have been collected in individuals with various diseased states. At the present time, insufficient data are available for any single disease to warrant conclusions.

Studies with Radiopotassium-42

Until recently, there was not available a single valid method for measuring quantitatively any constituent of the intracellular compartment. The introduction of radioactive potassium-42 suggested that a tool was finally available for such a study. There is available in the literature a study of findings of the "exchangeable potassium" content in a series of normal young individuals. Such a study has not been reported in individuals with various pathologic physiologic states. For the past two months, study has been in progress on the determination of the total potassium content of hospitalized subjects with various abnormal states. A total of 35 individuals have been studied to date. In most of these individuals, a significantly lower potassium content has been obtained than has been previously suspected in these diseases. More data will be accumulated in normal and abnormal states before any publication is prepared.

1090513

Studies in Normal Rabbits

Since the previous annual report was submitted, the data on fluid volumes and electrolyte concentrations in 45 normal rabbits have been summarized and published in the AMERICAN JOURNAL OF PHYSIOLOGY, 162:695, Sept., 1950. In this study, the results of the analyses of tissues and organs for their sodium-24 and water content, as well as a comparison of the thiocyanate and radiosodium spaces are given. This represents the first comprehensive study of this nature in normal rabbits, using artificial isotopes. It serves as a baseline for evaluation of the studies on pathologic states and establishes the validity of the methods.

Studies with Digitalis

It has not generally been appreciated that the digitalis glycosides which have been used for decades in the therapy of congestive heart failure are steriod compounds,, structually related to the adrenal cortical hormone and the sex hormones. The latter substances are known to affect salt and water metabolism. It is generally stated that digitalis has a specific cardiotonic effect; there has been some speculation as to the existence of an extracardiac or peripheral mechanism of action as well. In the treatment of circulatory failure in the course of acute infections, digitalis is sometimes used. The use in such diseases as pneumonia and Rocky Mountain spotted fever would be placed on a firmer basis if a peripheral action of its mechanism could be established.

In order to study this problem, hospitalized subjects without cardiovascular disease were given a therapeutic dose of digitoxin intravenously. Serial determinations were then made of fluid volumes. The data indicated that there was an increase in the water and protein content of the vascular compartment within 24 hours after the administration of 1.2 mg. of digitoxin. This alteration is difficult to explain solely on the basis of a direct myocardial effect. A summary of the findings has been accepted for publication in the PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE.

In a more detailed study of the same problem, a group of 7 normal male medical students were next given the same dose of digitoxin intravenously. In this series, urinary excretion of sodium and potassium and the alterations in the body electrolyte concentrations and contents were also followed; sodium-24 was used as a check on thiocyanate space. This experiment has been completed and the data are being analyzed.

Since there is some experimental evidence that toxic doses of digitalis cause a reversal of the effects of the usual therapeutic dose, rabbits were given a sublethal toxic dose of digitoxin and the changes in fluid distribution and electrolyte concentration and content, including sodium-24, were followed. The changes found could not be explained solely on the basis of a direct myocardial effect of digitoxin. It was concluded that large doses of digitoxin had caused cell damage and alterations in cell membrane permeability to sodium ion and water. The effects resembled those produced in animals by adrenalectomy or the administration of large doses of desoxycorticosterone. This work has been submitted for publication.

1090514

Comparative Study of Methods for the Determination of Plasma Volume

In previous studies on changes in capillary membrane permeability, the T-1824 (Evans Blue) dye method was used exclusively. Recently the iodinated serum fraction method has been introduced. From theoretic considerations, it would appear that this would be the ideal method for the clinical determination of the plasma volume or to detect alterations in capillary membrane permeability. Arrangements have been completed with Dr. D. L. Tabern of Abbott Laboratories to secure the iodinated plasma fractions from him. Preliminary comparative studies have been made in rabbits. Permission is awaited from AEC to use the material for clinical investigations in human beings.

George T. Harrell

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