ARMY MEDICAL SERVICE

RESEARCH PROGRESS REPORT

ANNUAL REPORT

1 JULY 1951 - 30 JUNE 1952

MEDICAL RESEARCH AND DEVELOPMENT BOARD
OFFICE OF THE SURGEON GENERAL
U.S. ARMY

30 June 1952
Reports Control
Symbol CSGLD-346

TABLE OF CONTENTS

Project No. 6-60-09-11
Contract No. MD-79

NOT FOR PUBLICATION
Report Date 30 June 1952

Serum Proteins in Liver Disease

Dr. Roy H. Turner
Tulane U. School of Medicine
New Orleans, Louisiana

BRIEF. The purpose is to investigate serum lipid and protein behavior in liver disease by means of an analytic centrifugation technique.

BACKGROUND. Experiments with P^{32} were carried out on 3 normals and 1 case of polycythemia. The rate of labeling of lipid phosphorus was especially rapid at some levels in the column of centrifugate and was low at others.

Utilizing the investigators method of using the quantity ultracentrifuge so as to sample the column at 10 levels and to make multiple analyses of the centrifugate, an experiment was devised to show the effect of complete caloric starvation in a healthy but obese medical student. Labeling with P^{32} indicated that the fall in concentration of neutral fat in the cream layer and increase in concentration of lipids in the low-medium density zone were associated with an increased rate of turnover of phospholipids in these regions. Tentative interpretation was that the lipids of the low-medium density zone play a special role in the adaptation of the individual to the stress of complete caloric starvation and that the lipids present in this portion of the centrifugate are in an as-yet-unidentified part of the path between lipid depots and the site where the fatty acids will be utilized in energy metabolism.

PROGRESS. The investigators accomplished the following: Devised a new and improved ultracentrifugal procedure for the study of serum lipids and proteins. This permitted finding for the 1st time of lipoproteins which contain phospholipids but no cholesterol; Established normal values for the influence of ingestion of lipid-rich meal, starvation, acute virus hepatitis, and other clinical disorders; determined differences in tagging with P^{32} at different levels in the ultracentrifugate; and described 3 systems of lipid complexes with the functions attributed thereto.

Project No. 6-60-09-11
Contract No. MD-80

NOT FOR PUBLICATION
Report Date 30 June 1952

Experimental and Clinical Studies on Acute and Chronic Hepatic Disease

Dr. Cecil J. Watson
Department of Medicine
University of Minnesota Hospital
Minneapolis 14, Minn.

BRIEF. To conduct studies which will correlate the physiologic dysfunctions encountered in infectious hepatitis and its chronic sequelae with the pathologic changes and clinical symptoms attributed to the infection.

BACKGROUND. Work on the effect of aureomycin on urobilin and stercobilin formation as related to changes in fecal flora yielded rather conclusive evidence that the reduction of bilirubin to urobilinogen occurs only in the intestine and requires the presence of coliform organisms, and that neither meso bilirubinogen nor stercobilinogen is formed in appreciable amounts following aureomycin therapy.

There was developed a quantitative microfluorospectrophotometer for cytochemical studies, especially of the liver. After offsetting the danger of too great sensitivity, valuable results from the work on Porphyrin Metabolism in Liver Disease are clearly indicated: a) Localization of porphyrins, ceroid, Rose Bengal, etc., can be readily studied. b) The study of fluorescence spectra of minute amounts of material in solution (i.e. = 10-13 gms. porphyrin) should prove valuable in characterization and identification.

Stimulated by Stich's report that riboflavin in large amounts markedly reduces porphyrin excretion, the effects of riboflavin phosphate given intravenously twice daily were studied. While very significant decreases of coproporphyrin were seen in certain cases showing clinical and laboratory improvement, the data are insufficient to prove these changes related to the riboflavin.

Studies on the Electrometric Method of Determination of Serum Cholinesterase. Dr. Frame and Miss Trainor completely confirmed the reliability of the method. Dr Stevenson collected data in various cases of liver disease in order to see whether the cholinesterase determination is of value in distinguishing hematemesis from a bleeding varix from that due to other causes such as peptic ulcer. It is also desired to learn its value with respect to liver injury from operations and anesthesia.

Studies on Experimental Liver Injury (Dr. Hoffbauer). Urinary and fecal copropor-

coproporphyrin frequently encountered in liver diseases are related to a disturbed cellular metabolism in the liver. Therefore substances known to have importance with relation to liver cell metabolism, have been used in large amounts and, wherever possible, in pure form. The broad plan has been to observe the patient's general status and liver-function tests done serially during a period of hospital diet and bed rest. In very ill patients, however, treatment was often started immediately and in these instances it was much more difficult and often impossible to assay beneficial effects, at least where there was improvement. Reasonably acceptable data have been recorded in 69 experiments conducted in 41 patients. A much larger number of patients was studied, but in instances of rapid improvement, rapid deterioration and death, or other factors, the data, not considered satisfactory, were omitted.

The selection and frequency of the laboratory procedures depended upon the character of the problem, with particular relation to the relative acuity and the presence or absence of jaundice. Usually the urinary coproporphyrin and urobilinogen were followed daily or every other day. The substances and number of experiments conducted with each, in the 41 patients follow:

Riboflavin (crystalline phosphate, 40 mgs. per day)	9	Coramine	1
Vitamin B_{12} (crystalline, 1000 mg. per day parenterally)	11	Folic Acid	4
Lipoadrenal extract (Upjohn)	8	Crystalline co-carboxylase (20 mgs. daily intramuscularly)	2
Aureomycin	12	Testosterone propionate	2
Chloromycetin	1	Necroton	2
ACTH	8	Intraheptol	2
Niacin	5		

There was some overlapping; a substance might be started while another was still being given. Since in these instances there was no evidence of therapeutic benefit, this factor might be disregarded, although the possibility of antagonism cannot be fully excluded. There is extreme difficulty in any event in controlling experiments of this type as to caloric intake, type of calories, fluid intake, activity, etc. It was perfectly clear in a number of instances, not included in the above group, that bed rest and hospital diet alone produced steady and often marked improvement. This was true only in the cases of alcoholic cirrhosis and hepatitis. The cases of cirrhosis following hepatitis or of idiopathic cirrhosis regularly failed to show improvement in any consistent fashion or in relation to anything that was done for them.

Of the above, those requiring mention are lipoadrenal extract, aureomycin, testosterone, and ACTH. The others, and lipoadrenal extract, had no significant effects from either a clinical or a laboratory standpoint. (This study of 8 cases failed to confirm Webster's claim that lipoadrenal extract was very beneficial in various types of subacute or chronic liver disease.) There was no benefit in 8 cases treated with ACTH excepting that in the 4 instances of cholangiolitic type in which pruritus was a prominent symptom and there was elevation of the serum bile acids, the exhibition of ACTH was followed by a significant decrease in the bile acid concentration and prompt amelioration or disappearance of the pruritus; this recurred in all instances within 2 weeks after the discontinuance of ACTH. In 1 instance 2 courses were given several months apart, with the same result on both occasions. The adverse effects included 2 instances of nematemesis following shortly upon the use of ACTH, 2 others of mental change, 1 of distinct increase of ascites and edema.

The effect of aureomycin was generally disappointing. Its administration to 3 patients in coma appeared to produce consciousness but the improvement was short-lived and the patients died. In certain cases of alcoholic cirrhosis, aureomycin eliminated the fever of cirrhosis and produced general improvement. No benefit was observed in the non-alcoholic, nonfatty group.

Experience with testosterone was very limited. Complete recovery followed its use in 1 case which appeared to be a long standing, subacute hepatitis, rather than cirrhosis. In the other case, clearly a post-hepatic cirrhosis, there was no improvement.

II. Studies of Stercobilin and Urobilin (with Drs. Green, Schwartz, and Clausen). A. Much attention has been given to the problem of an accurate, sensitive method of determining blood stercobilin. A very satisfactory fluorimetric method has been achieved for samples in which the bilirubin content is slight or negligible, but in icteric serums the oxidation of bilirubin to cholestelin interferes with the fluorimetry of stercobilin, as the cholestelin also gives a green fluorescence. Some very promising results have been obtained recently with methods which protect the bilirubin from oxidation during the formation of the fluorescing zinc complex of stercobilin. It is believed that this method will be valuable in further study of the stercobilin clearance in liver disease and in relation to the native urobilin and stercobilin of the blood in patients with hepatic functional impairment.

B. Dextrorotatory or d-urobilin has been studied intensively. In addition to its very great dextrorotation (-4000), it is characterized by an intense purple-blue color when heated briefly with dioxane and HCl. This color reaction is not given by stercobilin and is given to a very slight extent by urobilin IX, a - the latter being optically inactive and the former strongly levorotatory. A simple method of resolving mixtures of these 3 substances in very minute amounts semi-quantitatively will be applied to samples of urine and feces, with particular reference to the possible occurrence of d-urobilin in biliary tract infections. It is desired, furthermore, to study carefully the proportions of stercobilin, d-urobilin, and urobilin IX, a, in urobilin containing urines from liver and biliary tract disease and various hemolytic anemias in relation to: 1) the claim that mesobilirubinogen (urobilin IX, a) is formed in the liver rather than in the colon, and is not a precursor of stercobilin and 2) that very significant differences in the proportion of these 3 substances may be found in relation to certain types of liver or biliary tract disease. A much simplified method of isolating these compounds provides a much greater yield than any hitherto employed.

C. A study with Dr. Paul Lowry involves the isolation of N^{15} tagged bilirubin from the feces of a patient with hemolytic jaundice who was given terramycin. It was previously shown that terramycin at least temporarily affects the bacterial flora so that the reduction of bilirubin is markedly interfered with. Resistant strains soon grow up which can reduce bilirubin only to d-urobilin, not stercobilin. Dr. Lowry devised a new method for

easy approach to serial studies of fibrinogen in any given case of liver disease. The valuable data already obtained reveal a remarkable difference in blood fibrinogen content in certain cases.

IV. Serum Pseudocholinesterase. Further studies of the serum pseudocholinesterase in liver disease and in nephrosis have convinced the investigators of the value of this determination in following any case of diffuse parenchymal liver disease and have shown beyond doubt that in nephrotic hypoalbuminemia the value is normal or frequently elevated. This work, and Kelly's recent observation here, that there is a heightened rate of formation of albumin in cases of nephrosis, strengthen the concept now favored that the serum pseudocholinesterase level reflects the rate of albumin synthesis in the liver.

V. Studies of the Urinary Coproporphyrin in Liver Disease. A. It has been perplexing that the values obtained in liver disease with the new 5-ml. method appear to be relatively lower in respect to the normal range than might be anticipated. The mean normal value with the new method is 3 times higher than the mean with the old method, yet in a series of cases of liver disease the ratio appears to be closer to 1.5 or 2.1, and there are more cases with definite evidence of liver disease showing values within the normal range. This is unfortunate, and the reason for it has not yet been determined. The various factors are being studied.

B. The method of coproporphyrin isomer analysis is being studied with a view to finding a simpler and more nearly fool-proof modification.

C. A simple method has been developed for purifying porphobilinogen so that on heating it does not give rise to uroporphyrin. The uroporphyrin precursor is Ehrlich-negative in contradistinction to the porphobilinogen, which is Ehrlich-positive. This work revealed that the generally-held concept that porphobilinogen is a precursor of uroporphyrin is incorrect. This topic has direct application to liver disease, since porphobilinogen and large amounts of the colorless and non-fluorescing uroporphyrin precursor are present in the urine in porphyria hepatica, and it is now believed (see below) that in this form of the disease these substances are fabricated in the liver.

VI. The usefulness and sensitivity of the microfluorospectrometer have been increasing steadily. Developments included a new type of lamp housing for a more intense light source, and a chamber for cooling in an atmosphere of liquid nitrogen the tissue or material to be studied. Marked cooling sharpens fluorescence spectral lines, and this study shows that cooling allows the porphyrin fluorescence to persist much longer under illumination than at ordinary temperature. This is of special importance in relation to the relatively labile protoporphyrin. The apparatus has been very useful in the study of porphyria, in which the concentration of porphyrin in the tissues is high enough to permit easy detection even at ordinary temperature. Increased sensitivity was required, however, for the study of porphyrin fluorescence in normal tissues and in diseases other than prophyria. It is not yet certain to what extent this will be feasible, but it is believed that the apparatus has now reached a point of development where this is highly probable.

Partly by means of the microfluorospectrometer and partly with microanalytical methods of other types, work with Drs. Schmid and Schwartz has clearly established a basic classification of porphyria. There are 2 main types, porphyria erythropoietica and porphyria hepatica, based on the site of formation. In the 1st type the normoblasts of the bone marrow are clearly those cells in which porphyrin is forming and

it is evident that it is the nuclei in which this formation is greatest. In the hepatic types, however, the bone marrow is normal, and there is no evidence of porphyrin formation in the marrow greater than the normal slight amount (mainly protoporphyrin). The liver in these cases contains large amounts of porphyrin. In the intermittent acute form, however, this is present largely as a chromogen which does not fluoresce and therefore escapes detection unless the liver is first heated. In the mixed form of hepatic porphyria, characterized by photosensitivity and abdominal or nervous manifestations, the liver contains rather large amounts of performed porphyrin. This is probably fundamental with respect to the presence of photosensitivity in this group. The fact that in the cases of porphyria hepatica the porphyrin is excreted in the urine as a zinc complex is now taken to indicate that as it is formed in the liver it complexes with hepatic zinc. In porphyria erythropoietica, on the contrary, the porphyrin is excreted in the free state, probably because there is no zinc available for it to complex with in the bone marrow at the time of its formation.

VII. Studies of Experimental Liver Injury.

A. Attempts to Adapt Hepatitis Virus to Laboratory Animals. The influence of a yeast diet on the susceptibility of rats and hamsters to this virus was investigated. The inoculation of the virus was preceded and followed by brief periods of administration of ACTH or cortisone to enhance the likelihood of infection; results were negative. An attempt to infect cortisone-treated monkeys with suspected hepatitis virus was unsuccessful. In a study conducted with Dr. Lewis Thomas, 3 animals were inoculated.

B. Dietary Hepatic Necrosis in Rats. The study of massive hepatic necrosis in rats maintained on yeast-containing diets has been continued here. An initial goal has been the development of experimental conditions whereby massive necrosis can be recognized in an incipient stage. Once such conditions are established and known, dietary necrosis becomes a very useful tool for the study of post-necrotic scarring and cirrhosis. Very encouraging progress can be reported.

1. Bilirubinuria in Dietary Necrosis. Convenient methods are available for testing for the presence of bilirubin in small quantities of urine. When rats maintained on necrogenic diets are tested daily, 1/3 exhibit bilirubinuria as a terminal event and 1/3 exhibit intermittent bilirubinuria prior to death. Accumulated evidence indicates that the occurence of bilirubinuria is correlated with episodes of hepatic necrosis. It appears, therefore, that dietary necrosis is not always an immediately fatal event. Under the current conditions, some animals suffer an episode of necrosis, recover temporarily and succumb in a subsequent episode. All eventually die of a massive necrosis of the liver. It is now possible, however, to detect the occurrence of incipient or non-fatal necrosis in a certain percentage of animals and to study factors that influence it.

Study 2.) Effect of Addition of Tocopherol to Diet after Beginning of Necrosis (31 rats). It is known from the studies of Gyorgy and others that hepatic necrosis can be prevented if tocopherol or cystine is included in the diet. The present study indicated that tocopherol can prevent further necrosis even if it is added to the diet after an episode of necrosis has occurred. Tocopherol was given to 17 rats when the occurrence of necrosis was detected by positive urine tests. Four animals died of massive necrosis on the day treatment started but the remaining 13 survived 170 days. The untreated rats in the control group were all dead within 41 days.

Study 3.) Effect of Addition of Cystine after Beginning of Necrosis. (25 rats) This experiment is similar to Study No. 2; cystine was added to the diet when an episode of necrosis was detected. The study is still in progress; 11 treated rats (receiving 20 mg. of cystine per day) have survived more than 100 days.

Study 4.) Effect of Aureomycin after Beginning of Necrosis (50 rats). This experiment, which is similar to Studies 2 and 3, is still in progress. The results to date clearly indicate that aureomycin exerts a protective effect when added after an episode of necrosis has occurred.

2. Influence of a Synthetic Resin (Resion, National Drug Company). This substance had no influence on the course of dietary hepatic necrosis in rats. The survival time of the treated group (34 rats) was the same as that of the control group (34 rats).

3. Fatty Liver and Cirrhosis in Rats (Choline-deficiency). A study of iron metabolism (serum and liver iron content) with Dr. Robert Howard is nearing completion. A study relating to the pathogenesis of cirrhosis in fatty liver has been started. Specimens representing various developmental stages have been accumulated by means of biopsy, sacrifice, or death of 80 animals. The formation and behavior of regenerative liver nodules in cirrhosis have received particular attention. Since routine histologic methods are not adequate for this purpose, techniques involving blood vessel and bile duct injection have been used. Progress has been made in developing procedures necessary for this study. Two studies involving factors that might influence fatty cirrhosis have been completed: (a) Influence of aureomycin. Two groups of rats (35 in each) were maintained on an 8% casein diet; 20 mg. of aureomycin was added to the daily ration offered to 1 group. No significant differences between the control and the treated animals were observed. (b) Effect of substituting the necrogenic yeast diet after fatty liver or cirrhosis is established by a choline-deficient diet. In this experiments 12 rats were maintained on a choline-deficient diet for 85 to 138 days; the presence of fatty infiltration and cirrhosis was determined by biopsy. The daily ration was then changed to an 18% yeast diet known to be necrogenic.

Despite its necrogenic quality the yeast diet contains sufficient choline or choline precursor to cause virtually all the fat to disappear from the liver. The ceroid and fibrous trabeculae, when present, remain; the cirrhotic process initiated by the fatty infiltration does not progress; the regenerative nodules when present remain stationary. Finally, the characteristic necrosis occurs and kills the animal. The regenerative nodules seem just as susceptible to the necrotic process as the original hepatic tissue. Aureomycin delays the time of onset just as in instances where the liver is initially normal.

4. Other Experimental Studies. A histochemical technique suitable for recognizing necrosis of individual liver cells is very desirable. In a study carried out with Dr.

William Burnett, techniques employing tetrazolium compounds failed to yield the desired results.

An experimental study of the prolonged effect of heavy iron deposits in the liver is desirable. By appropriate diets, massive iron infiltration can be produced; the localization in the liver is peripheral; i.e. about the portal areas rather than the central as in the case of fatty infiltration. A short-term preliminary study (18 rats) of a modification of the corn-grit diet (Hegsted, Finch, and Kinney) was completed. The encouraging results warrant continuation.

A study (with Dr. Leon Cole) on the effects of parenteral aureomycin in rats having bile renal fistulas gave inconclusive results. Dr. Cole is now observing patients with various degrees of biliary obstruction. Duodenal intubation (Camus tube) is performed and samples of fluid secured. Aureomycin and bromsulfalein are then given intravenously; at intervals, samples of fluid are withdrawn from the duodenum and tested for the presence of bilirubin, bromsulfalein, and aureomycin. Such observations should be of particular value in patients with hepatic coma who respond favorably to parenteral aureomycin.

Project No. 6-60-09-11

NOT FOR PUBLICATION
Report Date 30 June 1952

Army Hepatic and Metabolic Center

Army Medical Service Graduate School
Walter Reed Army Medical Center
Washington, D. C.

BRIEF. The objective is to conduct clinical studies on acute and chronic hepatitis.

BACKGROUND. These investigations were moved from Valley Forge General Hospital in June 1950. Clinical, laboratory, and biopsy data on about 250 patients were placed on punch cards so that correlative changes could be quickly assayed.

ACTH in Acute Viral Hepatitis. This study on 31 March 1951 included 22 treated patients, selected in the 1st week of jaundice. Approximately the 1st 12 cases received ACTH in 1 dose daily subcutaneously for 7-10 days. Various courses have been used in a search for the best results without side-effects and without relapse upon withdrawal. The current method: 20 mgm. by slow intravenous infusion during each 12-hour period daily for 2 days; then 5 mgm. every 6 hours until patient has attained a normal serum bilirubin, when the drug is discontinued. The last 5 patients have been so treated with better results than seen in 9 controls.

Aureomycin and Terramycin Therapy in Chronic Liver Disease. Treatment of about 20 cases of chronic hepatitis and/or cirrhosis of the liver showed that these drugs produce a temporary fatty infiltration of the liver and a temporary elevation of BSP retention in a significant proportion of cases. Experiments were begun to see if a similar change could be detected in animals. One of the aureomycin groups was sacrificed at 30 and the last, 60 days after the start of the experiment. Liver sections did not show any convincing increase of fat in the aureomycin-treated animals compared with controls.

Liver Biopsies. Of the 340 biopsies performed so far, complications have occurred in but 2. In collaboration with AFIP, all of the tissue materials, including special patient-and-animal-study, are being stained for alkaline phosphatase, esterase, lipase, and desoxyribonucleic acid.

A Survey to Determine the Incidence of Hepatitis among Korean Casualties who have Received Irradiated as Compared with Unirradiated Plasma. Since 1 March 1951 this survey included Valley Forge and Percy Jones Hospitals. Of 400 Korean casualties surveyed at WRAH between September 1950 and June 15, 1951, about ½ had received plasma. Because of extensive wounds and the great amount of blood administered, the remaining half were also followed. In patients who received plasma plus blood the incidence of hepatitis was 21.6%. In those who received blood alone the incidence of hepatitis was 3.6%. "Hepatitis" represents jaundiced patients only. A month-by-month breakdown of the incidence of hepatitis reveals that the percentage of patients in the 2 groups is unchanged when comparing the beginning months of the survey with the final months. Since it is not as yet known whether this represents irradiated or unirradiated plasma, the survey will be continued.

Coagulation Studies (pilot) in Acute Hepatitis. showed that the 2-stage prothrombin time may definitely lag in its return to normal as compared with the 1-stage method of Quick. Some patients had normal 1-stage prothrombin times all through their disease and

at the same time had an abnormal 2-stage test. It seems in hepatitis an essential coagulation defect may be present which cannot be measured with the 1-stage method.

To date 330 determinations of the 2-stage prothrombin test have been made in 147 patients, including normals, Korean casualties, and patients with acute and chronic hepatitis, cirrhosis, obstructive jaundice, and miscellaneous diseases. The 2-stage prothrombin determination as a measure of liver function has these advantages: 1) if performed properly it is reasonably precise; 2) it appears to measure 1 single important liver function; 3) it is reasonably sensitive in acute hepatitis and in hepatic cirrhosis, although apparently not successful in chronic hepatitis; 4) it may be of some prognostic value in cirrhosis. Disadvantages are 1) it is not specific for primary liver disease; 2) it is not significantly abnormal in chronic hepatitis and 3) it is too difficult to standardize and perform in a routine fashion.

PROGRESS. ACTH Therapy in Acute Viral Hepatitis. To evaluate the use of adrenocorticotrophic hormone in the treatment of acute viral hepatitis, patients in the 1st week of jaundice were divided into 2 groups and alternate cases were chosen upon admission. Patients of the 1st group received ACTH intravenously along with 5% glucose and in the 2nd, control cases, intravenous glucose only was infused. A placebo is added to the glucose in the control group. The patients are all treated with the same basal regimen of bed rest until their serum bilirubin reaches normal. They are then permitted to be ambulated. All get essentially the same high caloric and high protein diet. Serial determinations are made of the serum bilirubin, the thymol turbidity, the Kunkel gamma globulin, serum proteins and the urine bilirubin. Data are then compared in the two groups.

The initial group of treated cases were compared with controls obtained when a study of aureomycin was being made. Later, alternate cases were selected as indicated above. Forty cases have been treated with various courses of ACTH; a few received subcutaneous ACTH. Later, combinations of subcutaneous ACTH and intravenous ACTH were being used and finally varying courses of intravenous ACTH only. The present course (and that believed most efficacious) consists of 20 mgs per day of ACTH for five days followed by 10 mg. per day for five days and then 5 mgs. per day until the patient attains a normal serum bilirubin. This ACTH is administered intravenously by slow drip. This therapy seems to result in a faster return to normal of well-being and of liver function tests. This prolonged and tapering course of ACTH seems to result in only a minimum of side effects, especially upon withdrawal of the ACTH. There seems to be little question but that the patients who received the ACTH have had a faster subjective response but the return to normal of liver function tests in the treated group over the controls seems questionable. In the treated patients mean values for the duration of positive liver function tests differ from the controls by about 1 week. These mean values may be misleading, since the range of values is such that there is considerable overlapping. In no test has there been a statistically significant difference. On the contrary experience with so-called "cholangiolitic hepatitis" seemed exceedingly encouraging. With the initiation of ACTH therapy in all such cases there has been a sharp drop in the serum bilirubin, which returned to normal within 10 days to 2 weeks.

Patients with metabolic endocrine problems and acute and chronic liver disease were studied serially to determine what pattern could be seen in the alpha-ketoglutaric and pyruvic acid metabolism under various forms of therapy.

This project is nearing completion. It has been found that where there is liver injury, acute or chronic, the alpha-ketoglutaric and pyruvic acid levels will be elevated in the blood. Patients with acute viral hepatitis studied during and after illness had an early rise with a return to normal of the keto acids and pyruvate. Pyruvate fell following institution of ACTH therapy. In patients who had portacaval shunts the alpha-ketoglutarate rose and remained elevated. In 2 patients who received aureomycin therapy, there was noted a sharp drop in the alpha-ketoglutaric acid with the onset of therapy and a rise to previous levels following its cessation. Conclusions may be drawn that the increased values for these substances indicate the presence of liver damage. Further experiments are being planned in connection with portacaval shunts and carbohydrate loads, and in patients otherwise under careful study.

Portacaval Shunts in Patients with Esophageal Varices.

Purpose: To evaluate an end-to-side portacaval shunt in the early treatment of patients with esophageal varices.

All patients with cirrhosis of the liver who have or have not bled from esophageal varices are esophagoscoped to determine if varices are present. If significant esophageal varices are demonstrated, the patients operability is studied. A group decision is then made as to whether he will have a portacaval shunt to prevent further or subsequent bleeding from his esophageal varices.

Eight patients have now been operated, of whom 7 had an end-to-side portacaval anastomosis and 1 a spleno-renal end-to-side anastomosis. One patient expired following surgery, done as an emergency procedure shortly after he started to bleed from esophageal varices. Although the postoperative course in a few of the patients was somewhat difficult, all other patients have done well. Experience by this team in the study of patients before and after shunt has led to the feeling that this is a procedure which should be used widely and especially earlier in the course of esophageal varices. No statistics are available with which to compare the longevity of the present group. It does seem certain, however, from the frequent follow-up esophagoscopies and the indirect measurement of the portal circulation time that the patients who have end-to-side anastomoses should not rupture varices from portal hypertension.

Measurement of Blood Citric Acid Levels.

Purpose: To develop a simple, sensitive microcolorimetric procedure for the determination of citric acid in blood, urine and tissue fluids which can be used for the differentiation of parenchymatous and obstructive jaundice.

The alkaline-pyridine method has now been established as an accurate and practical method for the determination of blood citric acid. Pentabromacetone is measured in a reaction mixture of alkaline pyridine which forms an intense red color on heating. The red color is measured to quantitate the content of citric acid.

Twenty-four determinations from twenty normal persons have established a normal mean value of 15.1 gamma per cc with a range of 8.1 to 18.9 gamma per cc. Eight of 9 patients with early acute viral hepatitis have shown elevated values. The exception was receiving ACTH. In 12 of 13 patients studied with convalescent or chronic hepatitis, normal values were found. Two patients with cholangiolitic hepatitis have been followed, one of whom had a persistent elevation of citric acid until ACTH was started. The citric acid then promptly fell to normal. Eight of 12 patients with portal cirrhosis were found to have elevations of blood citric acid roughly proportional to the clinical severity. One patient with hepatic metastases and another with secondary biliary cirrhosis were also positive. One patient with obstructive jaundice was found to have the lowest value which has been noted. This test is a promising one for evaluating the severity of hepatic disease.

A Comparison of the Effects of Fructose and Glucose on Nitrogen Balance. Two approaches were used: 1) a careful study under balance conditions on the Metabolic Ward and 2) empiric infusion of glucose and fructose in patients with chronic liver disease handled on a hospital ward. In the latter case only gross evidences of change in liver function will be noted. One patient with cirrhosis of the liver has now been studied with balance technique on a 48 day program during which he received glucose and fructose. Glucose was first given for 12 days followed by fructose and the cycle repeated. All carbohydrate was given by mouth.

Since the patient having the balance study was placed on a lower caloric intake than he had been receiving, a definite weight loss of 5 pounds was noted. After the first period of equilibration the patient remained in nitrogen balance. There was no certain evidence that fructose had any more beneficial effect upon this patient than did glucose. In the fructose period there were higher blood levels of the keto acids and other carbohydrate intermediates than in corresponding periods with glucose. Throughout the entire investigation the patient slowly improved from his liver disease. It was not believed, however, that the improvement was directly related to the ingestion of fructose or of glucose but to the mere passage of time. Another patient was treated with fructose who was not on a balance study but who also had decompensated liver disease. Here again improvement followed, but was not dramatic. This improvement could not be chronologically related to the fructose ingested.

Anion Fractionation of Urine. To elucidate the handling of certain electrolytes by patients with acute and chronic liver injury and related metabolic diseases.

By means of a cationic resin and differential precipitation both volatile and non-volatile organic acids, total anions, phosphate, sulfate and chloride of the blood and urine can be measured.

Data are being accumulated in a wide variety of metabolic abnormalities. This method is especially useful in special therapeutic regimes likely to result in acidosis

Blood levels of glucose and phosphate are determined by standard methods. Upon standardization of the preparation, constant glucose infusions will be given and blood samples taken at intervals from a peripheral artery and vein and from the hepatic vein. In addition, determinations of the blood lactate, pyruvate, citrate and alpha-ketoglutarate will be made. Hepatic blood flow will be measured by the BSP extraction method. The same animal preparations will then be given an insulin tolerance test. If the blood sugar response to insulin is decreased, the nature of the factors responsible will be further sought for by determining "insulin resistant factors" in the serum by the rat diaphragm technique.

Twenty animals have been studied. One fact seems to be emerging; namely, the carbohydrate intolerance in animals with fatty liver is not due to the inability of the liver to form glycogen.

Production of Hepatic Coma in Dogs.

Purpose: To produce in dogs hepatic coma comparable to that seen in advanced hepatic failure in humans so as to define the disease.

The initial goals have been achieved. The surgical techniques employed in the animals studied differed in 4 important respects from those which were prepared by Rappaport and Markowitz: a) an end-to-side anastomosis was done in these animals instead of a side-to-side anastomosis of the portal vein and inferior vena cava; the pancreaticoduodenal vein was ligated and the thoracic approach to the anastomosis was used, b) continuous glucose infusions were not consistently given, c) the diet used included meat occasionally, and the interval of time between shunt and hepatic artery ligation was slightly later in most cases, and d) the definition of "coma" used by the Toronto group was different. The results did not seem to be significantly different from those obtained at the Toronto Institute. These data proved to be very worthwhile despite the fact that this preparation finally is not strictly comparable with hepatic coma as seen in humans. Extensive biochemical studies made are being tabulated and will be carefully studied for application to human situations.

Albumin Binding of Bromsulfalein.

Purpose: To evaluate the binding of bromsulfalein by albumin and the conditions which affect it qualitatively in disease states.

Three approaches were used: the effects of: 1) increasing albumin concentration, 2) increasing BSP concentration and 3) the effect of albumin on the BSP spectral absorption curve.

Preliminary studies indicate that approximately 4 mgs of albumin bind 1 mg. of BSP (a molecular ratio of 2:1). This is in contrast to the reported binding with a molecular ratio of 1:1. It has been established that bound BSP is not colorless but gives a much less intense color than that which is not bound. Albumin shifts the spectral absorption curve and also depresses the maximum density. Attempts will be made to quantitate the ratio of free to bound BSP. It is the current belief that the decreased binding of BSP in jaundiced serum by albumin is not due to a defect in the albumin but possibly to competing anions which prevent binding of the dye.

Hepatic Vein Catheterization.

Purpose. To test the effect of various pharmaceutical preparations upon hepatic blood flow as determined by hepatic vein catheterization in anesthetized dogs.

A catheter is placed in the hepatic vein and with a constant infusion of BSP, determinations of hematocrit and BSP concentrations in peripheral vessels and in hepatic vein, a measurement of estimated hepatic blood flow can be made.

Since it was believed that the changes of hepatic blood flow seen with histamine and norepinephrine were related to changes in spleen volume, hepatic blood flow was studied in 13 splenectomized animals. Five dogs were given adrenalin, 4 were given histamine and 4 were given norepinephrine. The adrenalin had little or no effect upon estimated hepatic blood flow in the splenectomized dogs. Histamine produced a decrease and norepinephrine produced inconsistent changes of hepatic blood flow in these animals. There are no other promising materials available which might increase the hepatic blood flow as a possible therapeusis against shock.

In-Vivo Testing of the Citric Acid Cycle in Man and Animals.

Purpose. To measure in-vivo changes of the citric acid cycle under various conditions of loading with metabolic precursors and intermediates.

Many experiments have now been completed in animals where blood, urine, muscle and liver analyses have been made for components of the citric acid cycle after infusion of 2 carbon particles and 4 carbon particles. Such measurements include succinate, oxalacetate, alpha-ketoglutarate, pyruvate, and lactate. Where acetate and ethanol were infused there was seen an initial drop of blood pyruvic acid and a late small rise in alpha-ketoglutaric acid. This is interpreted to mean in the acetate experiments that the acetate reacts with tissue oxalacetate to yield citric acid which results in a temporary lowering of tissue oxalacetate. The tissue pyruvate reacts with carbon dioxide to supply the intermediate needed for oxalacetate, thereby drawing on the blood pyruvic acid to supply tissue pyruvic acid. This explains the fall in blood pyruvic acid which was noted in 30 minutes. Lactate and alanine can be assumed to be converted into pyruvate in dogs and used for formation of tissue oxalacetate. The equilibrium between oxalacetate and pyruvate seems to favor the pyruvic acid. A large rise of pyruvic acid, therefore, would lead to a smaller rise in tissue oxalacetate. This has been found where lactate and alanine have been infused. Also where ethyloxalacetate, fumaric, and succinic acids are infused there is a marked rise in alpha-ketoglutaric and pyruvic acids in the blood. In a single experiment in a human who had cirrhosis of the liver intravenous ethanol was infused and samples were taken from a peripheral artery, a peripheral vein and from the hepatic vein by means of a catheter. The data obtained are not subject as yet to interpretation. It is planned to repeat this experiment in another patient with

A modified method has been adapted successfully. It offers a primary advantage of rapidity with which multiple titrations can be done without the use of expensive automatic equipment.

In 2 normals, 3 patients with early hepatitis and 1 with cirrhosis, blood phenylalanine and trypotophane have been followed during a standard glucose tolerance test. In the normals a drop of both phenylalanine and tryptophane was noted in the blood following the sugar administration. Phenylalanine tended to fall more than did tryptophane. One diabetic and one patient with pituitary insufficiency showed a normal response. In liver disease this response was not noted. Following glucose administration the values for these amino acids were higher than in the fasting specimens. Plasma phenylalanine and tryptophane values were measured in response to exercise, insulin and adrenalin. In 2 normals and 2 patients convalescing from acute hepatitis a standard 10-minute exercise period on an adjustable treadmill was instituted. Blood samples before, immediately after exercise, and 20 minutes after exercise, were taken. In all the normals and in those with convalescent liver disease the amino acid levels rose markedly following exercise. After a 30-minute period of rest these values tended to fall toward normal. One-tenth of a unit of insulin per kilo was given intravenously to 1 normal, 2 patients with fatty liver and 1 with pituitary insufficiency. Samples were taker at 10- to 15-minute intervals. Insulin caused a marked drop in plasma phenylalanine and tryptophane almost immediately. They remained low throughout the experiment. The phenylalanine dropped more than did the tryptophane. When 0.5 cc of 1:100 adrenalin was given intramuscularly to 1 normal, both phenylalanine and tryptophane rose at the end of 10 minutes. These studies have great fundamental interest but must be temporarily deferred.

Iron Metabolism in Liver Disease. Intravenous Fe^{59} studies have been carried out on 5 normals, 8 patients at various stages of acute hepatitis and 12 with various hematopoietic disturbances. They were designed primarily to study total transport (plasma) iron turnover rates. It was hoped that a clue to the mechanism of the disturbance in iron metabolism (hypersideremia) could be found. In the various red blood cell dyscrasias it has been used primarily as a diagnostic tool. Results obtained in a) normals, b) acute hepatitis, and c) various red blood cell diseases:

a) 0.35 - 0.45 mgs per kilo per day of transport iron turned over (10 times the total plasma iron). 1.0% of the red cell iron (red cell mass) renewed, and destroyed per day - 7.0 - 8.0 gms hemoglobin produced and destroyed each day.

b) 0.5 - 1.5 mgs per kilo per day of transport iron turned over. 1.5 - 3.5% of the red cell iron (red cell mass) renewed (destroyed?) per day - 10.0 - 25.0 gms hemoglobin produced (destroyed?) each day.

c) Rapid turnover rate in various hemolytic anemias - 0.7 - 4.0 mgs per kilo per day, normal rate in a case of iron deficiency anemia due to chronic blood loss, slow rate in a case of aplastic anemia, and a slightly increased rate in a case of secondary polycythemia due to an A-V fistula.

Oral radioiron studies have been completed on 5 normals (3 with repeat studies) and 2 patients with siderosis (hemochromatosis) -1with a repeat study. In the normals less than 3.0% of the administered iron was absorbed when the iron was given as $FeCl_3$, whereas, in a single case given, the iron with ascorbic acid 7.0% was absorbed. In the 2 patients with siderosis 21.0 and 45.0% of the administered dose was absorbed..

As to excretion of the orally and intravenously administered radioiron in the normals, less than 0.01% of the iron was excreted in the urine and when the iron was given intravenously, less than 0.2% appeared in the stools. In acute hepatitis, a much larger amount of the intravenously administered iron appeared in the urine - 0.1 - 0.2%.

A new spectrophotometric procedure for the determination of the serum iron has been worked out which gives quantitative recoveries of iron from serum and requires only one ml of serum.

The study of iron metabolism in liver disease has established the existence of a hypersideremia in acute hepatitis, and has shown by the use of intravenously administered radioiron the presence of an increased turnover rate of iron (increased red cell production?). Radioiron studies in 2 patients with siderosis (hemochromatosis) revealed a marked increased absorption of oxidized iron. The evaluation studies of the serum iron procedure have led to the development of a more sensitive, reliable, and more quantitative procedure for its determination.

ACTH and Steroid Excretion. A few additional cases of liver disease given ACTH gave findings not significantly different from those reported. Some dehydroisoandrosterone assays have been done on some and the results seem to follow the same pattern as the corticosteroids.

The 1st series of urines obtained on front line troops in Korea have been analyzed but it is not possible to properly analyze the data. However, in some of the cases, high levels of the formaldehydogenic corticosteroids were obtained in the presence of a normal or low 17-ketosteroid excretion.

The method for the determination of the formaldehydogenic corticosteroids has been further evaluated.

The liver disease study has shown evidence that there is no hypoadrenalism in parenchymal liver disease, that the 17-ketosteroid excretion is low, but that these low levels are probably the result of a failure of the injured liver to convert the corticosteroids to 17-ketosteroids.

The studies on the procedure in use for the determination of the formaldehydogenic corticosteroids has shown up the many inadequacies of the existing procedures, and unless the procedure now under study, or a similar one, can be successfully used, the entire method should be dropped from use.

Electrophoretic Patterns. A very heavy demand has continued for electrophoretic analyses on patients at W.R.A.H. In all, 63 sera were analyzed during a 3-month period in the small electrophoresis apparatus. Only those samples involved in serious re-

Paper ionophoresis results were checked successfully with standard electrophoresis analyses. Successful preliminary experiments were conducted on electrophoretic convection apparatus. Over 10 runs were conducted with the promise of early application to difficult separations not possible with the conventional electrophoresis equipment.

REFERENCE. Peterson, Ralph E. and Mann, Joseph A. "Radioactive Iron Transport in the Intestinal Lymph", Am. J. Physiol., May, 1952.

Project No. 6-61-09-22

NOT FOR PUBLICATION
Report Date 30 June 1952

Studies in Immunization

Army Med Sv Grad School
Walter Reed Medical Center
Washington, D. C.

Envelope Substance of Pasteurella pestis

BRIEF. The purpose of this study is to determine the characteristics and biologic properties necessary for specific immunizing agents.

BACKGROUND. The Nature of the Envelope Substance of Past. pestis. Because of its potential value as an immunizing agent, the capsular substance of an avirulent strain of Past. pestis was studied. A simple casein hydrolysate-yeast medium can be used without employing the nutrient agar media previously used. In order to obtain good growth and capsule production it is essential to aerate this medium. The best method is provided by a specially constructed machine which rotates culture bottles while a stainless metal baffle, fixed in each bottle, creates extreme turbulence in the contained fluid.

Nonvirulent strains of this organism tend to dissociate into noncapsulated variants; search is underway to find the cultural conditions that will maintain the organism in a capsule-producing phase; success depends upon availability of large amounts of the capsular antigen. Chemical analysis of the purified capsular antigen is being carried out. Mild hydrolysis with acetic acid yields 2 components, not entirely distinct. The stability of the whole antigen to heat and chemical treatment was unexpected.

Pharmacologically Active Materials Associated with the Plague Bacillus. The fatal outcome in pneumonic plague suggests the effect of some toxic fraction or fractions of the organism. Death may ensue, even when adequate bacteriostasis is achieved, if therapy be delayed beyond the 1st day of disease.

At least 2 toxins isolated from avirulent Past. pestis are lethal to mice. Sonic vibration of the whole organism, living or dead, with or without capsule, releases a soluble endotoxin which produces widespread vascular lesions 1 or 2 days after injection into mice. These lesions are grossly similar to the necropsy findings of human septicemia and pneumonic plague, even to the extent of a hemorrhagic pneumonitis. Qualitative and quantitative data (preliminary) indicate that: 1) Cul-

gen from Past. pestis. Since the material has been clearly identified as a protein, the question of chemical purity is difficult to resolve. Important progress has been made in freeing the preparations from all but traces of contaminating nucleic acids, P content 0.1%. Chromatographic analyses for amino acids has yielded approximate values for some 12 amino acids with confirmatory data on 6 or 7.

Sedimentation rates and electrophoretic mobilities are important characterizing criteria. Best preparations to date show only 1 important boundary layer, indicating homogeneity of particle size and good progress in chemical purification. Molecular weight has been only approximated at 3,920,000. Electrophoretic mobilities were also approximated with further evidence for only 1 component.

The Toxin of Avirulent Past. pestis. Experiments were begun on the metabolism and the effect of the nutritional nitrogen, mineral, and carbon requirements on production of toxin and growth of the organism. Certain marginal media could not be used. Initial studies used a more defined medium containing casamino acids and yeast extract.

Attempts were made to grow the organism in completely synthetic media; results have not been consistent, but the organism will grow. Sodium desoxycholate which potentiates toxicity was used to extract the toxin. Results: a) Equal numbers of cells grown on media in which the concentration of casamino acids was varied do not necessarily contain the same amount of toxin. Subsequently, it is not the number of organisms per se which will give a certain amount of toxin, but factors in the cultural environment. An increase in total population did show a total increase in toxin production. b) Glutamine, previously shown to increase capsular production, but not growth, when added to 1% C-Y medium, showed no effect on toxin production.

Preliminary tests suggest that an excess amount of Fe, as $FeSO_4$, added to C-Y medium, slightly inhibits toxin production but not growth. However, varying concentrations of glucose appeared to have no effect on toxin production. Optimal concentrations of glucose will be established for increased growth.

Pathogenesis of Experimental Plague on Embryonated Eggs. Embryonated eggs inoculated via the allantoic space were used to study growth and toxin production of Past. pestis in an experimental closed system host. An avirulent culture of the TJW strain is in its 23rd egg passage. A comparison between susceptibility in relatively young and old embryos indicated the infection had a higher mortality in the younger embryos. There was a mortality of 95% at 35°C, 93% at 37°C, and 46% at 39°C.

To establish whether death of the embryo was due to a bacteremia or toxemia, a test correlated the numbers of bacteria with the amount of toxin present at intervals in the allantoic fluid of living eggs and of eggs which died. The plate counts showed that the bacterial count in eggs which died after injection increased on the 2nd and 3rd day of incubation and began to decrease on the 4th day. In these eggs, toxin was found at the time of the highest bacterial counts. Eggs which were alive had no toxin and showed much lower counts than did the dead eggs.

Antigen-Antibody Reaction and Relationships

BRIEF. The objective is to investigate the fundamental principles governing the in-vitro reaction of antigen and antibody. A quantitative study is to be made.

BACKGROUND. During preliminary incubation under the conditions of the complement-fixation procedure, complement is subject to various influences besides those proceeding from an antigen-antibody reaction. Possibly the most complex are associated with the test fluids under examination. Knowledge has been scanty and observations differed on the effect of such a common test fluid as human serum. These studies attempt to reconcile apparently conflicting evidence, and to determine the

factors in test fluid contributing to their complex effects upon hemolysis and complement-fixation. As a result of studies concerned with the influence upon C' of preliminary incubation with human serum and cerebrospinal fluid, it was possible to demonstrate the normal coexistence of enhancing and inhibitory functions in these 2 fluids, and to show their influence upon the standardization of the complement-fixation reaction.

PROGRESS. At the Department of Serology it was seen that the amount of complement (C') fixed by syphilitic serum reacting with cardiolipin antigen was not proportional to the amount of reactive serum present. Decreasing quantities of human syphilitic serum were tested, each with varying amounts of antigen and complement. After preliminary incubation at 3-6°C for 16-18 hours, hemolytic activity was determined by spectrophotometer. It was apparent that the relation of complement to syphilitic serum was not 1 of direct proportion. The C' requirement at high concentrations of antibody was considerably less than would be anticipated from the reactions with lesser quantities. Several explanations were considered.

A 2nd effect of nonantibody serum components was to increase the amount of antigen required for a maximal serum reaction. When the syphilitic serum was diluted in salt solution, maximal reactivity was elicited by antigen dilutions in the range 1:400 to 1:800. When normal serum was substituted as diluent, the strongest serum reactions occurred at dilutions of antigen of the order 1:200 to 1:400. Apparently this 2nd effect might result from the inhibition of complement-fixation rather than from separate causes. Thus, when normal serum served as diluent, an end-point reaction of the syphilitic serum fell at a considerably lower dilution of antibody, and the optimal dilution of antigen would be expected to undergo a corresponding decrease.

The Immunology Division reported the sedimentation constant of the human serum-antihuman serum saturated bi-antigen complex in which the antibody was obtained from the horse was found to be 9.7S. That of the bovine serum, antibovine serum bi-antigen complex in which the antibody was obtained from the rabbit, was found to be 8.8S. Calculations show that this difference in sedimentation constants is compatible with the antigen-combining sites on the horse antibody being on the same side of the molecule and close together. Similar calculations made for the bovine serum albumin complex show that the sedimentation constant is compatible with the antigen combining sites on the rabbit antibody being on opposite ends of the molecule.

The sedimentation constant of the egg albumin - anti-egg albumin bi-antigen complex in which the antibody was obtained from the rabbit was found to be 7.8S. This is compatible with the combining groups for antigen being at opposite ends of the molecule.

Parenteral Agents

BRIEF. The purpose is to reinvestigate the effects of dose, interval route, and other factors on immunization with parenteral agents in order to evolve more effective means of immunization. Under consideration is use of a multiple-dose high-pressure injection apparatus for mass immunization.

BACKGROUND. Development of High-pressure Multiple-injection Gun for Administering Biologicals. Compressed air and spring types were eliminated in favor of hydraulic pressure, 4 of this type having been built during 1949. A model tested in 1950 was sound in design but certain mechanical features needed altering.

Emphasis has been shifted to the assay and evaluation of commercial products used by the Services as immunizing and test agents. Experimental methods of assay developed under Fundamental Immunity (6-61-09-10) and Typhoid Fever and Asiatic Cholera (6-61-09-01) have been applied to routine assays of commercially prepared heat-phenol and acetone-killed vaccines. A method has been developed for the assay of the S. paratyphi and S. schottmülleri components in triple typhoid vaccines, both heat-phenol and acetone-killed.

PROGRESS. Using the Automatic High-pressure Jet Injection Apparatus, the 1st successful field trial was completed. Spring pressure had been increased from 180 to 230 pounds. Of 62 volunteers given standard issue typhoid vaccine, 59 were administered the dosage without difficulty. In 3 the jet failed at 1st to penetrate but did on succeeding attempts.

Assays and Evaluations of Products. Various dosages have been established whereby heat-phenol killed triple typhoid vaccines may be assayed by the mucin technique for ability to protect mice against challenge suspensions of the above organisms. From the ED_{50}'s obtained it generally appears that animals receiving triple typhoid vaccine and challenged with either S. paratyphi or S. schottmülleri exhibit a 2-3 fold greater protective titer than do those injected with a monovalent vaccine and challenged with its homologous organism. The method developed has been successfully applied to commercial typhoid vaccine submitted for assay of the S. typhosa component only.

Vaccine dosages for the assay of acetone-killed vaccines in CFW mice have been established. The ED_{50} in a susceptible strain of mice (CFW) is 4 to 5 times the ED_{50} in more resistant AfSGS mice.

Various experimental lots of acetone-killed monovalent and triple vaccines have been subjected to mouse protection tests. Certain lots have been examined for their ability to produce Vi antibodies when injected into rabbits.

Acetone-killed and dried organisms of S. typhosa, S. paratyphi, and S. schottmülleri were combined in the proportion of the proper nitrogen content in order to establish a new reference standard. This vaccine induces a Vi-antibody response in rabbits and is being subjected to repeated mouse protection tests to determine the normal range of ED_{50} values.

Tests to determine the effect of increased concentration of mucin on virulence-enhancing activity showed that a slight increase in concentration increased this activity. Also lots of mucin considered nonactive at the lower concentration become more active as the concentration increases.

Strains of mice from new sources were investigated in a search for an auxiliary source of typhoid-susceptible mice, but no other showed the same degree as that now used; however, assays showing graded response could be obtained at a differ-

ent level of protection.

To prepare a preserving fluid for blood vessels, the fluid was broken down into component parts which could be shipped without refrigeration and which would not deteriorate until the components were mixed at time of use.

Fundamental Immunity

BRIEF. The purpose is to investigate the basic mechanism of immunity through experiments that permit quantitative interpretation and statistical evaluation.

BACKGROUND. Studies of varied immunizing doses vs. varied challenge doses in mouse protection tests of typhoid vaccine showed the former was more efficient as a basis of measurement. Studies on the effect of various killing agents on the potency of typhoid vaccine showed merthiolate to be superior to heat-phenol or formalin. Later studies showed marked differences in the relative potencies of phenol-alcohol or acetone-killed vaccines, depending in part at least, on testing methods.

Glucose utilization was found essential for mouse virulence of S. typhosa, but not for Vi antigenicity.

Methods developed primarily for control and evaluation of potency tests of typhoid vaccine, but applicable to potency tests in general, were examined statistically for evaluation of the influence of such variables as mouse strain and sex, virulence of organism. Criteria for performing reproducible potency assays were defined.

The rate and specificity of immune response in mice injected with enteric vaccines were investigated. Specific immunity appeared in 24 hours and was almost maximal at 2 days in mice receiving typhoid vaccine. No such early response was apparent after injection of Shigella flexneri or cholera vaccines.

A study of the long-term response of rabbits to immunization by various routes and schedules was begun. Studies were begun (with the Veterinary Division) on the effect of pertussis vaccination on virus infections in mice.

PROGRESS. I Studies on rate and specificity of the immune response in mice were extended along these lines:

a) The paratyphoids: Mice immunized with monovalent S. paratyphi vaccine, either 2 or 6 days preceding challenge with the homologous organism, demonstrated high resistance at both intervals. Mice vaccinated with S. schottmülleri vaccine and challenged 2,3, or 6 days postimmunization with the homologous organism showed very slight protection at 2 days, whereas marked resistance was observed at either 3 or 6 days.

E. coli, 2, 6, 9 and 12 days prior to challenge with S. typhosa Ty2 suspended in saline, were found to possess essentially the same levels of immunity (ED_{50}'s) for Vi antigen.

3) Another test in which the immunizing doses were injected via the subcutaneous or intravenous route revealed no significant protection at 2 days, some at 4 days, while at 6 days there was no significant difference between protection by the various routes.

4) Essentially similar results, but quantitatively less significant (because of less steep dose-response slope) were had when mice were immunized with purified O antigen derived from S. typhosa O-901 and challenged with S. typhosa V 58 in mucin. When the O antigen was injected I.P. at intervals of 2,6, 12, and 18 days, the level of protection against challenge was essentially the same. Immunization via the subcutaneous route failed to disclose significant protection prior to 4 days after injection.

d) Re-evaluation of cross-protection, using smaller heterologous challenge:

1) Mice vaccinated with the ED_{50} dose of either typhoid, shigella or cholera vaccines were challenged with the homologous organisms and multiple LD_{50} doses of the heterologous organisms 2 and 6 days postimmunization. Those given the former showed no resistance to as low a challenge dose as 5000 Sh. flexneri 3 (1/100 of the standard challenge) in 5% mucin suspension. A possible slight nonspecific resistance was found at 2 days with 5000 V. comma, but no resistance was evident with 50,000 V. comma (standard challenge, V. comma - 500,000 organisms)

2) Mice immunized with Sh. flexneri vaccine exhibited no protection against any heterologous challenge at levels of 1/10 or 1/100 the standard challenge.

3) With cholera vaccine, evidence of a slight nonspecific resistance was also found against both 10 and 100 S. typhosa at 2 days; a possible nonspecific resistance against 5000 Sh. flexneri was seen at 2 days. However, none was found at 6 days with this challenge, or at either interval when challenged with 50,000 Sh. flexneri 3.

II Duration and level of antibody response:

a) Groups of rabbits, earlier immunized I.V. and subcutaneously with monovalent typhoid vaccines were studied to determine the rate and level of antibody formation. All rabbits were injected intravenously with a booster dose of 0.5 ml. of typhoid vaccine and bled at intervals over about 9 months. Prebooster O and H agglutination titers of rabbits, which had been vaccinated subcutaneously, were lower than those of rabbits given IV. doses, but titers of both groups were comparable 5 days following intravenous booster, remaining essentially so for 2 months. At the end of 3 months, agglutination titers approximated the prebooster levels, and in those surviving were essentially the same 264 days later. Thus the "residual" antibody level maintained after a long interval seemed related to the level attained by the earlier immunizing injections. At this time, an I.V. booster inoculation of 0.5 ml. was given; 7 days later, "H" and "O" agglutination titers of all rabbits were essentially equal, approximating the level of those found after the booster dose 9 months previously. The experiment was terminated.

b) Work on a satisfactory method for determining antibody nitrogen on these typhoid antisera continues in order to evaluate these results in terms more precise than agglutination titration.

III Cellular response of peritoneum to immunizing and infecting inoculations: Preliminary work has been done on the cellular picture of the peritoneal exudate in normal and vaccinated mice.

IV Quantitative immunochemistry of plague antigens:

a) The serologic activity of the purified "envelope" of Past. pestis discussed at the start of this report was studied in the quantitative precipitin test using a concentrated horse anti-plague serum from the South African Institute for

Medical Research. The optimal conditions for precipitation by this antigen were incubation of antigen and antibody for 2 hours at 37°C, then 5 days at 4°C. The precipitin reaction of this antigen with horse antiserum was characterized by a broad equivalence zone and an extended region of precipitation in excess antigen. Supernatant analysis indicated that the antigen was essentially homogeneous.

b) This purified antigen and the plague antigen (Fraction IA of Meyer) were studied in parallel in the quantitative precipitin test. Data comparing these 2 products reveal the essential immunologic identity of these plague antigens prepared by different procedures.

c) In studying the activity of the purified "envelope" antigen in the hemagglutination test, it was seen that human type "O" RBC treated with plague antigen in concentrations of less than 1 mg. per ml. of 10% cells failed to be agglutinated by anti-plague serum. Sera of rabbits immunized with the purified antigen and containing demonstrable antibody in precipitin and bacterial agglutination tests failed to agglutinate antigen-treated RBC. Only the most potent plague antiserum available (concentrated horse serum) caused clear-cut agglutination of the treated RBC. It was believed that such hemagglutination was due to an impurity or minor antigen, or that the "envelope" antigen had very little activity in this serologic test.

d) Rabbits were immunized with acetone killed and dried Past. pestis TJW strain and with purified antigen derived from these organisms. The total dosage employed was 1.9 mg. of organisms and 0.45 mg. of purified antigen. Agglutinin titers (employing viable suspension of Past. pestis TJW) ranged from 1:10 to 1:40 for both groups. The relatively low antibody content and limited quantities of rabbit sera available precluded the quantitative measurement of antibody nitrogen content.

REFERENCES.

McCallum, Edsall, and Carlson, "Rate and Specificity of Immune Response in Mice Inoculated with Enteric Vaccines." Bact. Proc., p. 112, 1952.

Edsall, Geoffrey, "Immunization of Adults against Diphtheria and Tetanus." Amer. J. Pub. Health 42: 393-400, April 1952.

Allergy following Vaccination

BRIEF. Possible allergenic or anaphylactogenic manifestations of vaccines are to be investigated.

BACKGROUND. Certain phenomena associated with vaccines prepared from viruses grown on embryonated eggs have been studied. Conclusion: there is a substantial potential danger in using such vaccines in the crude state. Several means of partial purification of virus preparations have been devised. Such methods permit removal of from 75 to 95% of the host tissue substances, including a number of substances

In no instance has this treatment been sufficient to effect the complete inactivation of the virus suspensions studied, but results have been sufficiently promising to justify further investigation.

Chemical Composition of the Chick Embryo. The study of nucleotide composition has been continued. This study will provide data concerning the rates of production of ribonucleic and desoxypentosenucleic acids in the normal chick embryo.

A rapid and efficient process for purification of nucleotide extracts has been devised. It involves the removal of low-molecular-weight impurities with the use of a finely-divided neutral adsorbent such as Attaclay SF. Extracts of chick-embryo nucleotides thus purified have been examined in the ultracentrifuge and electron microscope. Preliminary data indicate that the polynucleotide particles are greater in molecular weight than 1×10^6 and have axial ratios of 300 to 700.

Immunology of the Major and Minor Blood Groups

BRIEF. The objective is to utilize quantitative hemagglutination techniques to explore the energy of binding of isohemagglutinins with their homologous red cells.

BACKGROUND. Recent work by Fillite, Wurmser et al. indicated that it is possible to secure quantitative thermodynamic data in isohemagglutination which should shed light upon the mechanism of red cell agglutination. But they were unable to establish absolute values of the reagents involved in their reactions. This project will apply quantitative immunochemical techniques to the problem.

In the routine assay of sera for red cell typing not only is the absolute titer of a given serum significant, but its "avidity" is of paramount importance. Avidity refers to the tendency of cells to clump and is measured by the speed of clumping of red cells when reacted with a serum of a given titer, and the size of the clumps. It is hoped that more objective and quantitative measurement of avidity will be made possible.

PROGRESS. Preliminary tests were directed toward familiarization with the technique of quantitative blood counts, recording counts photographically. The techniques of the French workers mentioned were checked in regard to incubation of cell-serum suspensions, shaking, etc; results were duplicated. An improvement was devised; it consisted of shaking during incubation of the cell-serum suspension. The last experiments demonstrated a significant drop in cell counts in the controls without added serum.

Fate of C^{14}-labelled Antigens

BRIEF. The purpose is to investigate the biochemical and biophysical forces involved in the organism's defenses against injurious factors.

BACKGROUND. Use of caterial proteins labelled with C^{14} as antigens was discarded because of lack of a workable method for securing a single pure protein. Pneumococcal polysaccharides have been isolated in antigenic form. Such a medium has been defined.

PROGRESS. Preparation of Bacillus friedlanderi by 3 different techniques gave products of poor antigenic qualities. Further work with this organism has been abandoned. More recent work resulted in definition of a synthetic medium suitable for growth of the Pneumococcus; 15 liters of this medium have been inoculated and harvested to allow for polysaccharide isolation.

FUTURE. Substrates to be used: 1) uniformly C^{14}-labelled d-glucose, 2) C^{14}-labelled CO_2, 3) C^{14}(Methyl)-labelled acetate, and 4) an hydrolysate of Myco. phlei labelled with C^{14}.

Project No. 6-59-08-04
Contract No. MD-99

NOT FOR PUBLICATION
Report Date 30 June 1952

Radiation and Thermal Burns

Dr. Everett I. Evans
Medical College Hospital
Richmond, Virginia

BRIEF. This project was designed to investigate, separately and in combination, the radiation, traumatic, and thermal types of injuries, expected to result from atomic explosions, and to develop optimum and practical methods of treatment with emphasis on handling of mass casualties.

BACKGROUND. Fluid and Electrolyte Requirements in Severe Burns. A simple formula for intravenous fluid therapy of the burned adult during the 1st 24 hours after injury was tried in Richmond hospital cases studied. The extent of the burn, the presence of respiratory burn, the age of the patient, and the general state on admission are the chief factors affecting his recovery.

In burns of small extent (10-20%) it was seen that the baby or very young child suffers burn shock earlier and more intensely than an adult similarly burned. Consequently, all babies and children with burns more extensive than 10% of the body surface are given shock therapy as soon as possible. A patient with a respiratory burn from inhalation of irritating gases may early show signs of pulmonary edema which itself can be fatal, despite shock therapy. Colloid and salt administration in these cases is restricted because they develop pulmonary edema quite readily soon after, during, or even before intravenous infusions of large amounts. Patients over 50 must be examined carefully for cardiovascular renal disease, or overloading of heart and kidneys may occur. Patients seen the 1st time more than 1 or 2 hours after severe burning may be in a state of moderate to severe shock; prompt vigorous shock therapy is usually required.

PROGRESS. I. Clinical Burns Unit. In the past year, based upon observation of 115 hospitalized patients, 47 white and 68 colored, there was a remarkable improvement in the death rate from severe burning injury. Provided adequate care is given, 2 factors are of paramount importance in prognosis: age (above 50 years) and extent of burn (50%).

Over 4,095 days' hospital care was furnished these patients. The burning agents were: fire, 56%; scalds, 25%; hot metals, 11%; miscellaneous, 8%. Of the 115 admissions there were 19 deaths of 16.5%. The average extent of body surface

III. N^{15} Anemia Studies. Utilizing the N^{15}-tagged red cell technique, there was found a marked reduction in hemoglobin synthesis following burning in the dog as well as a dynamic difference in response after burning injury. Those red cells produced after burning injury appeared to have a normal life span. A similar study has been completed in 1 burned patient. Precise measurements of stercobilin production were made in the burn patient for correlation with life span of newly produced cells. Similar tagged fresh red blood cells have been infused in 1 normal to permit comparison of life spans of infused cells.

IV. Bacteriologic Study of the Burn Wound. The burn wounds of 82 patients have been cultured. The trends seem to be:

The local use of antibiotics, while reducing the incidence of overall infection, may simultaneously induce the development of resistant bacteria.

Despite adequate antibiotic therapy, cross-infection of the burn wound is becoming increasingly serious when a central dressing station is employed.

Preliminary data indicate that the presently available antibiotics, although they seem to diminish the severity of invasive infection, will by no means solve the problem of burn wound infection.

V. The Stress Response in Severe Burns was studied in 21 severely burned patients. A full-scale adrenocortical study was made on each over the 1st 10 days. The quantitative eosinophil counts reflect quite well the adrenocortical function of the burn patient. The depression of the eosinophil count was greatest and longest in large burns, least in small burns; the stress response varies with the severity of the burns. Quantitative balance studies of urinary corticoids, nitrogen, Na, K, and water in these patients confirmed this conclusion. The adrenocortical response to thermal injury may be conceived as a sort of massive internal inflammatory reaction. The administration of ACTH and Compound F to normals (unburned) confirmed this conclusion and supported the contention that DOCA-like compounds may be released after thermal injury. Clinical trials of ACTH and cortisone in the shock phase of severely burned patients did not promise much gain in survival in such patients. Pseudodiabetes, similar to that found in Cushing's disease, was discovered in severely burned patients who had been on a high carbohydrate, high caloric intake soon after burning injury. It has not been found so commonly since the carbohydrate level of intake has been reduced.

VI. Nutrition in Burns. 1) Studies of the Plasma Ultrafiltrate in burned rats indicated a) an increase in the taurine fraction, b) a large amino acid conjugate increase, and c) a large increase in histidine or histidine-like compound. Of greatest importance is the conjugate amino acid fraction which on hydrolysis yields glycine in greatest amounts.

2) Intravenous Fat Emulsions were employed in too few cases to warrant any definite conclusions.

3) Nutritional Requirements of Burned Patients. Fifty burned patients have been studied metabolically. Although the dietary requirements of the severely burned adults appeared much higher than those of normals, it is still not known how much higher. The diets have consisted almost entirely of liquid feedings; fat emulsions were the chief source of calories in 40 of these patients. It has been

found possible to give fat at high levels to the severely burned patient. The nutritional requirements of the burned child appear better known from this study.

4) The Influence of Exercise on the Injured Patient. These metabolic studies showed that planned active bed exercise for the injured limbs reduces greatly the nitrogen loss in the traumatized individual. Total body weight loss in the exercise group was considerably less than that in the control group.

VII. Experimental Burns in Animals. Extensive studies were made on combined thermal and radiation injury in dogs in an attempt to simulate the injuries that might be inflicted by an atomic bomb explosion in the 6,000 to 7,000-foot zone. Dogs were given the standard 20% contact burn injury complicated by the addition of 100r external body radiation. This small amount of external body radiation resulted in mortality of 73% as contrasted to a mortality of 12% when the burn alone was inflicted. All dogs that died as a result of the combined injury had a blood stream infection of beta hemolytic Streptococcus, type G and L. When this same experiment of combined injury was carried out, except that the animals were treated with penicillin, the mortality dropped to around 18%, and no animal developed a blood stream infection of beta hemolytic Streptococcus.

REFERENCES. Dr. Evans and his co-workers published these reports:

"The Early Management of the Severely Burned Patient", Surg., Gyn. & Ob., 94: 273-282, March 1952.

"Fluid and Electrolyte Requirements in Severe Burns", Ann. of Surg., 135:6, 804.

"Atomic Burn Injury", Medical Aspects of Civil Defense, Council on National Emergency Medical Service, AMA, Chicago, Ill., June 1952.

"A Standard Contact Burn", Surg., Gyn. & Ob., 93: 543-554, November 1951.

"Nutritional Problems of Patients with Burns", Nutrition Rev., 9:257-260, September 1951.

Project No. 6-59-12-21
Contract No. (Navy Contract)

NOT FOR PUBLICATION
Report Date 30 June 1952

Thermal Burns

Dr. Oliver Cope
Mass. Gen. Hospital
Boston, Mass.

BRIEF. The objective is to study the local burn and its exudate in order to determine the basic derangements and to develop local therapy. Homologous skin grafts as a temporary life-saving measure are to be investigated and metabolic studies, using radioactive tracers are to be made.

BACKGROUND. Funds for this cooperative study were given the Navy in September 1950. The Office of Naval Research reports under "Intracellular Changes in Trauma Depletion and Repair".

Ointments for Dressings.were studied. Carbowax has a more retarding influence than petrolatum.

ACTH. The survival of homografts in guinea pigs and young hogs was not lengthened by the use of cortisone or ACTH. No evidence was found that ACTH diminished the need for plasma colloid, electrolyte, and water therapy of the burned patient in the initial 36 hours. Burns treated with ACTH developed measurable edema and blebs of the same extent, duration, and protein concentration as comparable wounds in untreated patients. Patients with active Cushing's disease due to hyperfunctioning adrenal cortical tumors, who volunteered for controlled experimental burns, developed the same edema and blebbing as did normal volunteers. No difference in edema or blebbing was seen between ACTH-treated and nontreated members of pairs of young hogs given controlled burns.

Using the Drinker dog-foot lymph preparation, ACTH did not influence edema formation, or rise in flow and protein concentration of lymph.

PROGRESS. Between 1 January 1951 and 15 May 1952, 151 burned patients were cared for.

I. The Wound.

Two completed projects explored the use of homografts in treating of full-thickness burn wounds. Homografts offered the most physiologic dressing for a full-thickness burn wound yet devised. The survival of homografts proved unpredictable; no means were found

internal administration.

II. Shock - Fluid Therapy.

Effect of Adrenal Hormones on Capillary Permeability and the Need for Fluid Therapy. The claim that ACTH given to the patient would diminish the need for fluid in the shock phase has been extensively investigated and no substance to the claim has been found. Apparently it was based on a misinterpretation of an effect of ACTH on the infectious inflammation which appears as a later phase of the burn wound. Fluid is needed in the early shock phase of the burn because the capillaries are damaged by heat leak plasma fluid in excessive quantities, the fluid puddling in the burn wound as edema. Blood volume fails and dehydration of the rest of the body occurs if fluid is not promptly administered intravenously. With the passage of time and the growth of bacteria in the wound this phase of capillary damage due to the heat passes into one of infectious inflammation which may also be associated with abnormal capillary leakage. This infectious phase is not significant in fluid therapy until 2 or more days have passed, the time depending upon the infecting organism.

Use of Radioactive Chromium in the Measurement of the Circulating Red Cell Mass. This method of tagging red cells offers the best method thus far found. The chromate ion forms a firm bond with the red cell without destroying it. The bond is formed within a half hour. The chromium attaches itself to the globin. If the red cell is hemolyzed by a burn, it sticks to the globin in the plasma. The concentration of chromium in the plasma can thus be used as a measure of hemolysis following a burn in an animal injected with chromium-tagged red cells prior to burning. This method seems much better than the radioactive phosphorus method.

Experiments have been done on dogs. A burn of 50% of the body surface in water of 85 - 90° C for 30 seconds hemolyzes an average of 10% of the red cells. Less severe burns hemolyze fewer cells, more severe, deeper burns a greater number. The percent of cells hemolyzed has been checked by giving a 2nd injection of chromium-tagged cells. The difference in volume between the 1st and 2nd chromium volume should also equal the volume of red cells destroyed. There is good correlation between the 2 determinations of red cells hemolyzed.

This method was also used to measure the red cell mass in patients. The volumes found in well patients believed to have a normal red cell mass agree with the radioactive-iron-tagged cell method. In the only 2 severely burned patients seen only minor losses of red cells due to the burn were found.

It was the initial impression that the proportion of the red cell mass destroyed by the average burn was not therapeutically significant. Whole blood therapy is not mandatory in the initial hours unless the burn is truly a deep, cooking, extensive burn. This situation should be distinguished from the later need to make good the progressive anemia. More such observations are needed.

Blood Viscosity in the Critical Evaluation of Whole Blood Therapy. A study here of the viscosity of both the whole blood and of the plasma following a burn and its relation to a rising hematocrit (in the dog and in the burned patient) showed that the viscosity also parallels the hematocrit in burns. The rise in viscosity should be associated with slowing of the circulation in the undamaged tissues and

organs, with reduction in the nutrition. How far the diminished oxygenization resulting from viscous blood can be offset by the increased oxygen-carrying capacity of this blood with its greater hematocrit remains to be seen.

The observations thus far showed that flow of the more viscous blood to the brain is reduced, but with the increased oxygen-carrying capacity the oxygen consumption of the brain remains close to normal. As long as the brain can withdraw all the oxygen from the blood with greater than normal hematocrit, the disadvantage due to diminished blood flow is offset. These experiments in blood flow are tricky and continued investigation is indicated.

Edema Volume and Need for Fluid Therapy - Radioactive Sodium Versus Thiocyanate in the Measurement of Extracellular Space. Under normal conditions the thiocyanate and radioactive sodium volumes are almost identical. Observations here indicate that under acute stress, such as burns and perforated ulcers, the 2 volumes depart widely from each other, the sodium being considerably larger. During widespread inflammation in the later phases, 2 spaces are reversed. Study of these 2 spaces may yield important data regarding the passage of certain ions through cell membranes.

Re-evaluation of Formulas for Fluid Therapy. Workers here are recalculating experience with extensively burned patients to see how four formulas now in use in burn therapy stack up.

III. Metabolism

Ten severely burned and 3 moderately burned patients were observed from the time of injury through healing of their wounds. The metabolic rate of 6 patients without burns who were undergoing major surgical procedures were observed.

Following extensive thermal trauma the metabolic rate is elevated, plus 30 to plus 60, for as long as 2 months; it gradually recedes to normal as the wounds heal.

In circumscribed burns involving but 15 - 20% of the body surfaces, the metabolic rate is normal or only slightly elevated.

In the patients undergoing major surgical procedures the matabolic rate rose slightly for the 1st days after operation. The basal metabolic rate of the non-burned volunteer was not significantly altered by a high protein diet.

The protein-bound iodine of the blood serum and the thyroid uptake of a tracer dose of I^{131} were measured in 7 patients. The measurements, specific

Acute Bone Decalcification and Calcium Poisoning of the Kidneys. It is believed that in 1 burned youth studied, his 6-months immobilization plus the high calcium intake of the milk diet had induced these complications - convulsion, impairment of renal function, with albuminuria and elevated NPN.

Project No. 6-59-12-22

NOT FOR PUBLICATION
Report Date 30 June 1952

Traumatic Surgery and Shock

Army Med Sv Grad School
Walter Reed Army Medical Center
Washington, D. C.

BRIEF. The purposes are: to study the mechanisms, physiologic effects, and control of hemorrhagic and traumatic shock, and to develop practical and improved surgical and chemotherapeutic methods for handling such patients.

BACKGROUND. Liver Function during and Following Anesthesia and Surgery. To determine the effects of various anesthesias, duration of anesthesia, and surgery upon liver function, patients are selected from the operation schedule. Blood samples are taken preoperatively, after induction of anesthesia, and at intervals following surgery. A panel of liver function tests are performed;and estimations are made of the blood keto acids by means of paper chromatography and plasma prothrombin by a 2-stage method in a few surgical patients. The 25 patients studied included 10 with major thoracic and abdominal procedures. In none was there liver disease prior to surgery. There was no obvious influence upon the liver function tests exerted by operative trauma or anesthetic agents in otherwise normal individuals. On the other-hand, in patients with chronic hepatic disease undergoing extensive surgery there seemed to be a marked response to the trauma of surgery and anesthesia in all of the liver function tests measured. Although extensive, these changes were not permanent and values returned to their previous (or improved) levels within 2 weeks.

The Use of the Artificial Kidney in Metabolic Problems. One patient with an hepatorenal syndrome was dialyzed for 2 hours. Then it was necessary to stop because of gastrointestinal hemorrhage. Definite chemical benefit was obtained. A 2nd patient, terminal with a fulminant hepatitis, developed anuria and severe potassium intoxication with intermittent gastrointestinal bleeding. Although chemical and electrocardiographic improvement resulted, he expired from his primary illness.

One patient, dialyzed twice for potassium intoxication arising from acute anuria, responded well but expired with pulmonary edema after 23 days of anuria. It is believed that with prolonged anuria of this type irreversible tissue changes take place which are not affected by dialysis therapy. Another patient who developed anuria following a transfusion reaction was dialyzed because of potassium intoxication. He developed a spontaneous diuresis and made an uneventful recovery. A patient suffering with chronic uremia was dialyzed with the usual chemical changes being effected

the plasma and blood volumes, the amount of circulating plasma albumin and globulin, and the effects of closure of the fistula on these measurements. Studies have been made of the kinetics of mixing of T-1824 and I^{131}-albumin with the circulating blood, the possible dissociation of the test material from its protein-bond, and its possible loss into the lung during its 1st passage. The latter possibility has also been studied with Na^{24}. The arterial dye-injection curve was completed in 7 pre-operative fistulas, 8 postoperative fistulas, 11 controls, and 3 subjects before and after manual occlusion of the fistula.

Experimental Shock. The volumes of distribution of T-1824, Na^{24}, and D_2O are under study in dogs before and during experimental hemorrhagic shock.

Although unexplained, there is evidence that different vascular responses result from specific kinds of shock-producing injuries. Instrumentation and techniques were perfected for study of these factors:

1) total body hemodynamics, which includes central and peripheral arterial pressures, cardiac output, and circulatory competence; 2) cardiogenic factors, including heart rate, coronary anoxia, cardiac stimulation, the strength and possibly the rate of cardiac ejection; 3) venous return from the upper and lower parts of the body; 4) peripheral resistance, including measurements of "total" and regional vasomotor tone as well as reactivity to arterenol; 5) measurements of the size of the effectively supplied vascular bed (T-1824); 6) the effectiveness of oxygen transport in relation to demands of the body; and 7) tests of sympathetic and parasympathetic activity.

Design of a Flame-Photometer was perfected.

The role of bacterial infection in shock was 1st studied in blood cultures from dogs with and without portacaval shunts subjected to hemorrhagic hypotension. Bacteria were cultured occasionally, but not consistently.

1) Guinea pigs have been inoculated with coliform bacteria and their toxins and with extracts of dog feces and then challenged with the same antigen after 4 weeks. It has been possible to induce anaphylactic shock in guinea pigs sensitized and challenged with sonic-killed Gram-negative bacteria. Several others developed severe but sublethal allergic manifestations. 2) Mouse toxicity studies were done by injecting mice with plasma from dogs in various stages of hemorrhagic shock. No toxic effects were seen.

Dogs subjected to hemorrhagic hypotension have been treated with the reinfusion of shed blood by either arterial or venous transfusion. Results in this small series fail to indicate superiority of arterial transfusion except when the heartbeat has ceased. Under those circumstances arterial transfusion has been successful in resuscitating more animals than the venous route of transfusion.

The Surgical Research Laboratory located in WRAH is doing analyses of acid-base and fluid and electrolyte balance problems.

Surgical Research Team in Korea. Studies on water balance with D_2O have begun; adrenal steroid excretion, electrolyte balances, and dehydration studies are under way. The team acquired an artificial kidney for trial; plastic blood transfusion bags are being evaluated.

PROGRESS. The Role of Bacterial Infection in Shock. The role of endogenous bacterial infection in shock is being studied. The permeability of the intestine to bacterial toxins in shock and control states has been studied by means of a known bacterial toxin as a tracer in 15 pairs of dogs. Of each pair one dog is put into shock and the other serves as a control. Botulism toxin has been instilled into the gastro-intestinal tract and the hourly blood samples have been assayed for toxin content by means of mouse toxicity. Preliminary results indicate that there is a greater level of this toxin in the blood as a result of shock than in control dogs.

Arterial and Venous Blood Transfusion in Shock. Cineroentgenograms were made at the Army Medical Research Laboratory, Fort Knox, Ky., on the fate of diodrast given arterially and venously in rabbits in shock. While this product is far from satisfactory it served in developing a teaching technique.

Fluid and Electrolyte Changes Associated with Trauma. The Surgical Research Laboratory at WRAH is continuing to do analyses of acid-base and fluid and electrolyte problems in the hospital. It is serving as a focal point for analysis of physiological problems for WRAH.

Experimental Shock and Circulatory Mechanisms and Fluid and Electrolyte Changes. The Walcott technique, which most closely resembles uncontrolled shock due to hemorrhage in man, has been used in animals anesthetized with nembutal. Findings at gross autopsy are identical to those found in other types of experimental hemorrhagic shock. The over-all physiologic pattern includes: 1) cardiac and respiratory acceleration, 2) decline of arterial pressure, 3) coronary anoxia, 4) hyperthermia, 5) alterations of pulse contour, all eventuating in respiratory failure. The decline of pressure is not countered by significant limb vasoconstriction. Nonetheless flow is diverted away. The circulatory collapse occurring is not due to a body-wide loss of sensitivity to pressor agents nor to bacterial processes. There is a wide range of individual susceptibility and circulatory response.

Measurements of blood volume, of fluid, protein and cell mobilization and loss, and of permeability of the vascular compartment have been found in considerable error. Experiments have shown these concentrations are profoundly influenced by volume and distribution of blood flow, factors unquestionably altered in shock, but completely neglected previously. New measurements of these functions have been developed which are applicable to all types of shock.

Therapy of circulatory collapse in shock by vasopressor drugs may augment the deleterious effects of reduced renal blood flow. Two such agents, epinephrine and norepinephrine, are being studied in normal dogs. Incomplete results indicate an increase of flow at low dosage, a decrease at high levels by both drugs. In general

T-1824 when administered simultaneously.

A case of renal tubular acidosis with nephrocalcinosis is under study; the condition has been shown for the first time to be familial.

Biochemistry of Connective Tissues. This project was initiated several years ago to obtain fundamental biochemical data relative to rheumatic fever and to normal as well as other pathologic states of connective tissue. More than 20 publications include: the preparation and purification of hyaluronidase and hyaluronic acid; analytical methods for hyaluronidase and serum anti-hyaluronidase; kinetics of the enzyme reaction; the dependence of serum anti-hyaluronidase on Mg, its relation to scurvy in guinea pigs, its relation to rheumatic fever, and the effect of salicylates in vitro; the effect on capillary permeability; the effect of rutin and anti-histaminics; and the collagen content of some connective tissues.

The best combination of published techniques for preparing hyaluronic acid from umbilical cords has been incorporated into a simple, reproducible method. Analytical data show a high degree of chemical purity as well as high viscosities, indicating limited degradation of the polymer.

Plasma Pulmonary Clearance During the First Circulation. The Stewart Principle has been modified for the evaluation of net exchange of micromolecular solutes across capillary beds. Flow measurements based upon the mean dilution of an indicator during its first circulation will be artefactually elevated if a portion of the indicator is diverted from the stream during that circulation. Hence, if a crystalloid is administered simultaneously with a colloid, differences in blood flow determined separately from their concurrent dilutions must reflect the net extravascular (i.e. transcapillary) loss of the more diffusible agent.

This technique of simultaneous indicators was applied to a study of net salt and water transfers in the lesser circulation of man. T-1824 was employed as the intravascular standard. Such use of T-1824 was validated by the demonstration of instantaneous and complete dye-protein union during the 1st circulation.

T-1824 in various combinations with I^{131}-labelled albumin, inorganic I^{131}, Na^{24} and D_2O was administered to 24 subjects. Delivery was made through a catheter within the subclavian vein. This method did not disclose any significant transcapillary exchange or extravascular dilution of electrolytes within the lesser circulation of normal man.

The T-1824/Na^{24} flow ratio in a patient with mitral stenosis and proven pulmonary hypertension was 0.902. In another with a large carotid arteriovenous fistula, the T-1824/Inorganic ratio was 0.881. Both values suggest transcapillary electrolyte exchange. Preliminary values in 4 subjects with deuterium indicate that even the exchange of water is minimal within the lesser circulation of normal man. Results obtained in peripheral capillary beds are strikingly different than the above. Thus 40-60% of inorganic I^{131} crosses the capillary in a single circulation through the lower extremities of both man and dog. This amply attests to the sensitivity of the simultaneous indicator technique. The fate of white blood cells in the lung has also been studied by this new method. Leukocytes have been separated from blood by fibrinogen flotation and labelled with P^{32}.

Kinetics of Simultaneously Administered T-1824 and I^{131}-Labelled Human Serum Albumin. This project has been subdivided into 2 categories:

The early mixing and late metabolic rate of T-1824 as evaluated by the simultaneous administration of T-1824 and I^{131}-labelled human serum albumin: The first circulation was isolated from subsequent phases of T-1824 mixing by application of the Stewart principle. Simultaneous transit of T-1824 and iodinated albumin was evaluated in 2 vascular beds of widely different pressure and capillary relationships, i.e. the lung and the lower extremities. In seven subjects transit of T-1824 through the heart and the lung in the 1st circulation was identical both for T-1824 and iodinated human serum albumin. In 3 separate determinations identity of transport during the 1st circulation with these 2 indicators was also demonstrated in the lower extremity. The evidence would indicate that virtually instantaneous and complete union of T-1824 to albumin occurs immediately subsequent to intravascular introduction of the dye. This effectively refutes any of the reports of early reticulo-endothelial abstraction of unbound dye anions. The promptness of dye-binding does not necessarily reflect the stability of the resulting dye-protein complex. Therefore the late simultaneous degradation of T-1824 and I^{131} albumin was assessed in 10 human subjects. The indicators were administered simultaneously by separate injections from separate sites. Measurements were extended over a period of 1 week. Plasma decline of indicators during this late phase presumably represents pure metabolic turnover rather than mixing phenomena. T-1824 and I^{131} albumin disappeared at virtually identical rates and distributed over approximately equal compartments. Wherever divergence occurred it was in the direction of greater loss of dye but this never exceeded 10-15%. In addition, 24-hour concentration of dye was determined in 27 subjects. Disappearance of dye averaged 50% during mixing of 1 day. The experiments would indicate that T-1824 approximately mirrors albumin transport.

Artefactual divergences of distribution compartments resulting from mixing of T-1824 and I^{131}-labelled albumin prior to injection: Different results than the above are obtained if T-1824 and I^{131} albumin are mixed together in the same injection vial in concentration wherein there is marked excess of the dye. In-vitro this manipulation does not change the properties of the iodinated albumin. Electrophoretic mobilities and ultracentrifugal patterns are unaltered. Butanol-extractable I^{131} and dialyzable I^{131} fractions do not increase. Moreover, despite the incubation of dye with iodinated albumin, the chromatographic R. F. image of the radioactive material remains the same. Nevertheless subsequent to administration of these premixed indicators a rapid (3-8 minutes) abstraction of 10-15% of the iodinated albumin occurs in vivo. This divergence has been demonstrated in 35 humans as well as 3 dogs. The metabolic removal of a portion of the iodinated albumin is permanent and discontinuous. Thus over a period of 1 week in 3 subjects there was

REFERENCES. "Simultaneous Determination of Plasma Cardiac Output with Evans Blue and Iodinated Albumin," Freinkel, Schreiner, and Athens. Amer. J. Physiol., 167: 785, 1951.

Pulaski, E. J. "The Clinical Status of Dextran, PVP and Gelatin,", Chem. and Eng. News, 30: 2187, May 26, 1952.

RESEARCH AND DEVELOPMENT PROJECT CARD (NEW PROJECTS)	2. SEC. U	3. PROJ. NO. 6-59-12-024
1. PROJECT TITLE: Plasma Volume Expanders		4. REPORT DATE 30 June 52
6. BASIC FIELD OR SUBJECT: Surgery	7. SUB FIELD OR SUBJECT SUB GROUP: Therapeutics	AVF-6
8. COGNIZANT AGENCY: The Surgeon General, DA	12. CONTRACTOR AND/OR LABORATORY: See below	CONTRACT/W. O. NO.
9. DIRECTING AGENCY: Med Res & Dev Bd		
10. REQUESTING AGENCY	13. RELATED PROJECTS	17. EST. COMPL. DATES: RES. Cont.
11. PARTICIPATION AND/OR COORDINATION		DEV.
		TEST
	14. DATE APPROVED 7 Jan 1951	OP EVAL
	15. PRIORITY 1-C 16.	FY 18. FISCAL EST'S.
19.		52 482M
		53 341M
		54 405M

20. REQUIREMENT AND/OR JUSTIFICATION

Intravenous fluids to expand the circulating blood volume will be urgently required in large amounts for atomic casualties. Whole blood and its cellular elements cannot be stockpiled because of short storage life and insufficient supply. Plasma, albumin and other derivatives are also subject to limitations of supply and possible transmission of homologous serum hepatitis. Stable, safe, effective, cheap plasma volume expanders which can be stockpiled in large quantities are urgently needed.

21. BRIEF OF PROJECT AND OBJECTIVE

a. Brief: The objective is to investigate the characteristics, physiological effects, safety, and efficiency of the gelatines, oxypolygelatines, dextrans and polyvinyl pyrrolidones as plasma volume expanders. To develop new and more effective plasma volume expanders.

In the event of atomic warfare a mass of casualties suffering from shock incident to burns, traumatic wounds and hemorrhage will present an immediate problem of profound magnitude. The use of blood and blood derivatives to expand the circulating blood volume in the treatment of shock is a well-established surgical principle. Available supplies of blood and blood derivatives to meet this emergency would be insufficient. It is, therefore, imperative that a suitable product or products be developed, which can be stockpiled for use as plasma volume expanders in the event of an emergency.

The idea of preparing and utilizing solutions of compounds or material having the colloidal and osmotic characteristics of plasma is not new. Despite previous efforts to develop completely satisfactory plasma volume expanders, none have been found which are entirely free of disadvantages. The difficulties involve the selection of a foreign material that will provide the desirable characteristics of human plasma and be completely metabolized without producing toxic or allergic reactions in the recipient. From the standpoint of practical use, a plasma volume expander should also have sufficient stability to permit storage, distribution, and administration under various environmental and emergency circumstances. The availability and cost of manufacture are factors which also require consideration.

22. JRDB	SY.	PC.	IC & P.	Y.	I.	C.

JRDB FORM 1A, 1 APR 1947

Several potential plasma expanders, notably Dextran, PVP and various gelatines, are presently available but there are many unsolved problems connected with each of them.

Dextran, obtained from fermentation of cane sugar was developed in Swedon after the last war and has been used extensively in Europe for the treatment of shock. The molecular size of the product may vary widely, which is undesirable. Efforts must be made to produce a product in which the molecular weight is controlled between narrow limits. After intravenous administration most of the Dextran disappears from the blood stream in 12 to 14 hours. About one-tenth to one-half is excreted in the urine, but the fate of the remainder is still incompletely determined. Tracer studies will be done to investigate this matter more completely. Toxic effects have not been observed, but allergic reactions have occurred which must be investigated.

Polyvinyl pyrrolidone (PVP) was introduced in Germany during World War II and was used extensively as a plasma volume expander. Like Dextran, its molecular size varies and its metabolic fate is unknown. It is non-antigenic. Although promising, PVP needs much more study to determine its comparative value as a plasma volume expander.

Various gelatins have had wide use in this country as plasma volume expanders. P-20, an osseous gelatin has been thoroughly studied and found to be effective, but it has limited practical use because of its gel state at usual room temperatures. Oxypolygelatin, a chemically modified gelatin, shows some promise but much investigation is still required.

Since all present potential plasma volume expanders have disadvantages, efforts should also be made to develop new and more effective plasma volume expanders.

b. Approach: (1) Characterize both chemically and physically all plasma volume expanders for the purpose of developing inspection and control methods. (2) Attempt to modify the various plasma volume expanders to improve the shape of their molecules and to control molecular weights within narrower limits. (3) Develop analytical methods for measuring the plasma volume expanders in body tissues, fluids and excreta. (4) Determine the effects of the presence of plasma volume expanders in the body on the reliability of standard methods for blood analysis and on measurements of cell and plasma volumes. (5) Investigate changes in plasma volume expanders on aging in storage. (6) Produce radio-active tagged materials for tracer

reticulo-endothelial system and other vital organs. Those products found to be safe and effective in animals will be similarly tested in normal human subjects and those suffering from shock. (9) Attempt to develop new and more effective plasma volume expanders.

The ultimate aim of this project is to produce a stable, safe, effective, cheap plasma volume expander which can be stockpiled in large quantities for use in case of an emergency.

Par. 12 Contractor and/or Laboratory

a. Surgical Research Unit

Will test plasma expanders in animals and human military subjects, with particular attention to safety, reactions, efficiency in restoring and maintaining blood volume, value in correcting shock, and practical value for military use. Will investigate the metabolism, excretion, retention and storage of plasma expanders, and their effects on the kidney, reticulo-endothelial system, and other vital organs.

b. Emory University - Dr. W. L. Bloom

Will study the clinical effects of dextran on the dyhamics of circulation and on kidney function. Will investigate the hydrolysis of dextran by body tissues and extracts, notably by muscle, liver and spleen; and by body enzymes, notably 1 - 6 glucosidase. Will perform tracer experiments, using dextran labelled with radioactive carbon - 14, to determine excretion, storage, and metabolic breakdown in the body. Will evaluate dextran in clinical patients, for shock and for its effects on patients with liver and kidney diseases. Will develop analytical methods for dextran in body tissues and fluids.

c. University of Pittsburgh - Dr. T. S. Danowski

Dextran and PVP (polyvinylpyrrolidone; Periston) will be studied in animals which have the salt-depletion type of shock. Their effects on circulation will be measured by cardiac catheterization, ballistocardiography, blood pressures, cyanide circulation time, and changes of kidney function. Following this, similar clinical studies of dextran and PVP will be made on patients suffering salt-depletion and other forms of shock.

d. University of Iowa - Dr. S. M. Horvath

Will study, in animals in hemorrhagic shock, the effects of plasma expanders on cardiac output, liver blood flow, renal functions, arterial and venous blood pressures, blood volume body water, body weight, appetite, and the composition of

the blood. Animals will be followed at least two weeks to determine rates of return of functions to normal. Later the effectiveness of the plasma expanders in dehydration and environmental stress (heat and cold) will be investigated.

e. Yale University - Dr. L. G. Welt

Will investigate the effects of plasma expanders on the metabolism and excretion of water and electrolytes in normal human subjects and in edematous patients. Will use iso-tonic solutions of the plasma expanders, but which are either iso-oncotic or hyper-oncotic. Will determine whether the plasma expanders are capable of inducing diuresis in edematous patients. Will study the utilization of chromium tagged red cells for measurement of blood volume and sulphur-35 in the measurement of extracellular fluid volume.

f. Ohio State University - Dr. R. M. Zollinger

Will do comparative evaluations of the plasma expanders in human patients for the treatment of shock due to trauma, burns, or hemorrhage. Observations will be made of clinical response, local reactions, systemic reactions, and effects on blood pressure, plasma volume, cardiac output, plasma osmotic pressure, blood coagulation, blood viscosity, blood flow, hemolysis of red cells, kidney functions, electrolyte balance and acid-base balance. The fate of the infused expanders will be investigated; retention in the circulation and storage in the reticulo-endothelial system; rates and molecular sizes excreted through the kidney.

g. Harvard University - Dr. J. Fine

Tracer studies in animals will be performed with PVP (Periston; polyvinylpyrrolidone), labelled with one or more of the following tracer tags: N-15, C-14, I-131. Quantitative determinations of the distribution, excretion and deposition of PVP will be made by analysis of tissues, blood, urine, feces, and expired air, using radioactive techniques and the mass spectrometer. Isotope analyses will be checked where possible with chemical analyses for PVP and with histological examinations of tissues. The effects of PVP on blood volume, fluid balance and electrolyte shifts in normal and shocked animals will be observed. Functional damage to tissues and organs will be measured, and the toxicity of PVP will be determined in mice and rats. Attempt will be made by plasmapheresis to replace plasma with PVP to determine its potential for injuring tissues. If the tracer studies are not conclusive, additional labelling of the aliphatic chain of PVP

rates and constants in the ultracentrifuge. Will develop a new method to obtain these results in a shorter time than the one week now required. Will determine the changes in molecular sizes of gelatins in solution which occur during storage.

i. University of Pennsylvania - Dr. H. M. Vars

Plasma expanders will be compared by standardized animal tests which were developed for evaluating intravenous gelatins. Values for expansion of plasma volume and correction of hemorrhagic shock will be measured quantitatively. Rates of excretion in the urine and disappearance from circulation will be measured. Toxicities will be determined. In collaboration with the Knox Gelatin Company (Dr. D. Tourtellotte), attempts will be made to produce and evaluate oxypoly-gelatins with gel points below 50° F. Clinical trials of expanders, which appear safe and effective by animal experiments, will be made in human patients.

j. National Academy of Sciences - Dr. F. D. Lawrason

Will serve as a central information office for all phases of the plasma expanders program, providing "up-to-the-minute" information to investigators, committees of the National Research Council, agenices of the Department of Defense, and potential manufacturers. Will arrange the production and distribution to investigators of all plasma substitutes, including tagged materials for tracer studies. Will duplicate all research reports from investigators and distribute them to appropriate agencies of the Armed Forces and National Research Council. Will assist the National Research Council committees in the analysis of research data and preparation of reports, as a basis of recommendations by the National Research Council and implementations by the Department of Defense.

k. National Bureau of Standards - Dr. S. G. Weissberg

Will characterize, chemically and physically, the plasma expanders, for the purposes of developing inspection and control methods, which will serve as the basis of satisfactory purchase specifications, adequate inspections, and sound product controls. A series of practical analyses will be developed, which will insure that types of products wanted can be specified to industry and procured by the government. Osmotic pressures, light scattering, intrinsic viscosities, sedimentation rates, and fractionations will be investigated. Changes in plasma expanders on aging in storage will be investigated. Possible modifications of dextran, to improve the shape of its molecules by chemical treatment, will be attempted. Radioactive dextran for tracer studies will be characterized.

l. New York University - Dr. R. C. Warner

Will develop analytical methods for measuring the plasma expanders in body tissues, fluids, and excreta. Will determine the effects of the presence of plasma

expanders on the reliability of standard methods for blood analysis and on measurements of cell and plasma volumes. Will develop analytical methods for metabolic intermediates of plasma expanders found to be metabolized in the body. Will develop analytical methods to chemically characterize the fractions of plasma expanders supplied by manufacturers.

m. Columbia University - Dr. A. Cournand

Will establish emergency teams, on call at both Bellevue Hospital and New York Hospital, to treat traumatic cases with plasma expanders, and to make thorough clinical studies of each case from the time of admission. The protocol will be that of D. W. Richards and A. Cournand, developed in 1944-45, for the study of concentrated albumin solution as a plasma expander. Particular attention will be paid to hemodynamic effects.

n. Carnegie Institution of Washington - Dr. L. B. Flexner & Dr. F.P. China

The permeability of the capillary beds in various organs and tissues, to the plasma expanders will be determined with tracer tagged materials and instantaneous dynamic methods. Storage in tissues will be determined at various times after injection. The effects of plasma expanders on the blood volume and the distribution of body water between the vascular and extravascular compartments will be determined.

o. St. Vincent's Hospital - Dr. L. M. Rousselot

Will investigate the dehydrating effects of the plasma expanders and measure the water displaced from tissues into the circulation, in normal human subjects and patients in shock. Will measure the amounts of proteins displaced from the circulation and tissues by plasma expanders.

p. Polytechnic Institute of Brooklyn - Dr. F. R. Eirich

Will study the molecular characteristics of PVP (Periston; polyvinylpyrrolidone) solutions, to include distribution of molecular sizes, molecular shapes, extent and types of branching, solubility, viscosity, membrane permeability, osmotic and oncotic pressures, light scattering, sedimentation velocities, interaction with cosolutes, and behavior as a polyelectrolyte under physiological conditions. These data will be used, as far as practicable, to define PVP in purchase specifications

r. Children's Medical Center, Boston, Massachusetts - Dr. E.R. Blout

Will synthesize water-soluble high polymers from naturally occurring alpha-amino acids for possible use as blood plasma expanders. After synthesis, the pharmacological and physiological properties of each product will be tested.

s. Washington University - Dr. Robert Elman

The effect of various plasma substitutes including dextrans will be studied both in animals and in patients. The patients will be those admitted to the Barnes Hospital and St. Louis City Hospital and Homer G. Phillips Hospital who have developed evidence of surgical shock following operation, burns, tissue injury or massive hemorrhage. In addition to the usual blood chemical studies, special techniques will be developed for the measurement of the colloidal osmotic pressure and for blood volume determinations. The latter involves the use of a new carbon monoxide inhalation technique.

t. Army Medical Service Graduate School

Will attempt to develop methods which are more suitable for the micro-analysis of dextran in blood. Will study the reaction-inducing properties of clinical dextrans and the etiology of these untoward reactions.

u. University of Pittsburgh - Dr. Paul H. Maurer

Will conduct studies relative to the antigenic activity of Dextran. The study will involve the quantitative determination of antibodies to both the high and low molecular weight dextrans in various sera collected at Brooke Army Hospital. In addition to the antibodies to dextran a quantitative estimation of the antibodies cross-reacting with dextran will be made. Passive transfer and sensitization studies are also expected to be done.

v. Sloan-Kettering Institute for Cancer Research - Drs. Leon Hellman and David Becker

Will study the behavior of various C^{14} labeled plasma expanders in the body to include their distribution in the various body fluids and tissues. Because of the feasibility of measuring small quantities of plasma expanders with the isotope technique, the behavior of tracer quantities of these materials can be compared with that of full therapeutic amounts.

w. Cornell University - Dr. James M. Neill and Dr. Edward J. Hehre

The immunological properties of "native" and of partially hydrolyzed dextrans

will be studied using a variety of products from the Northern Regional Research Laboratory, "native" dextran from other sources and commercial products prepared for clinical use. The occurrence and reacting capacities of non-dextran constituents of dextran-forming bacteria in culture fluids will be determined. After appropriate methods are developed, the examination of clinical dextrans for the presence of trace amounts of serologically reactive non-dextran constituents of bacterial origin will be done.

x. Emory University - Dr. Paul B. Beeson

To determine the relationship between molecular size and Shwartzman activity of hydrolysis fractions of dextran and correlation with leukopenia-producing capacity. It will be determined if tolerance develops for dextran's Shwartzman adivity as it does for the activity of other bacterial products. Should tole develop, further studies would consist of a chemical measurement of rate of disappearance from blood stream as tolerance develops, the measurement of dis pearance rate in animals made resistant by repeated injections of bacterial pyrogens, plus a study of the effect of so-called reticulo-endothelial blockad on this tolerance.

y. California Institute of Technology - Drs. Linus Pauling and Dan H. Campbell

To conduct chemical studies of oxypolygelatin to provide a basis for improvement of the product and for interpreting the findings of biological evaluation studies. New chemical and physical methods for reducing the gelation tendency of gelatin will be sought.

d. Other Information: None.

e. Background and/or Progress: There is no substitute for whole blood in the treatment of mass casualties during a national emergency. Inability at present to preserve whole blood beyond 3 weeks makes it impossible to build stockpiles of this material. Plasma and plasma substitutes must make up the difference between supply and demand. Stored pooled plasma has not yet reached the point of absolute safety from transmission of disease. A standardized human globin solution is not yet available. The material currently available is still capable of producing untoward reactions.

have the disadvantages of solidifying at low temperatures, thus interfering with blood typing after infusion. Polyvinyl pyrrolidone, of large molecular size, is retained indefinitely in the body while smaller molecules are rapidly excreted so that their functions as plasma expanders are limited. Dextran, like gelatin and polyvinyl pyrrolidone, is still to be controlled from the standpoint of molecular size. Certain preparations are apparently antigenic and may cause allergic reactions. Other disadvantages are the possibility of harmful effects on certain tissues or organs and the fact that end products are not likely to contribute to the synthesis of new body proteins.

Gelatin and Gelatin Products

(1) Gelatin has been subjected to comprehensive study in the past 10 years, and 6 percent solutions have been found to be both safe and effective as a plasma expander when given intravenously for the treatment of early stages of traumatic shock. This solution of gelatin is composed of relatively long molecular chains which may be retained in the circulation for as long as 4 days, and shorter molecular chains excreted by the kidney shortly after infusion so that their osmotic effects are only transitory. No significant damage to vital organs has been noted either in animals or humans receiving gelatin. There is evidence that some of the retained gelatin is metabolized. Solutions of the material are stable. The practical advantages of gelatin are its availability, low cost, and freedom from reactions. The disadvantages are: (1) the smaller molecules are not retained sufficiently long in the body; (2) difficulty with blood typing and cross-matching is encountered after infusions because of pseudoagglutination of the red blood cells, for which reason it is advisable that samples for blood matching be taken before a gelatin infusion is given; and (3) use in outdoor conditions is greatly limited by the fact that gelatin is in the physical state of a gel and at low temperatures plugs or runs slowly through intravenous equipment. Gelatin solutions that remain liquid at low temperatures have been produced and are on the market, but they consist of small molecules that are rapidly excreted and thus do not exert the same efficiency in the treatment of shock as do the larger molecules. Gelatin has the further disadvantage of being an incomplete protein and so does not contribute materially to the formation of new protein.

The gelling problem appears to have been largely overcome by the development of oxypolygelatin, and without sacrifice of molecular size and osmotic properties. Oxypolygelatin is prepared from the parent substance by decalcification with an ion exchange resin, condensation with glyoxal, oxidation with hydrogen peroxide, and sterilization by autoclaving. A 6 percent solution, which has a mean molecular weight of 40,000, remains liquid at temperatures down to 10° C. The product is nonantigenic. After infusion it has about twice the osmotic activity of an equivalent volume of plasma. The problem attending oxypolygelatin is the control of molecular size. Clinical trials have not been extensive, but results in

treatment of early traumatic shock parallel those of gelatin.

Polyvinyl Pyrrolidone

(2) PVP is a colloidal polymer of acetylene of large molecular weight. It was developed in Germany as a plasma expander. A 3.5 percent solution in Ringer's solution has a mean molecular weight of about 40,000. PVP can be rapidly produced in large quantities from readily available materials. It is estimated that more than 500,000 intravenous injections were given to German soldiers in World War II with satisfactory results. PVP is stable on storage in the dry form and in solution and is nonantigenic. It is about equal in effectiveness to plasma and gelatin in restoring diminished blood volume resulting from hemorrhage in the experimental animal and in man. The ultimate fate of PVP in the body is now undergoing investigation. From 20 to 50 percent of the amount infused can be acco for in the urine in the unchanged state, and traces can be detected 10 days a injection. Experiments with radioactive materials have shown that less than 1 percent is exhaled as carbon dioxide in the first 10 days following adminis tion. These experiments further demonstrate that the nonexcreted portion -- molecules having a molecular weight greater than 31,000 -- is taken up by the reticuloendothelial system, the skin, and the skeletal muscles, and stored fo undetermined length of time. Whether this storage will produce harmful effec is not yet determined. On the other hand, if the molecular size of PVP is reduced below 31,000, it is rapidly excreted by the kidneys and its function as a

Work in progress at Walter Reed Army Medical Center suggests that dextrans contain sufficient nitrogenous products to cause the appearance of precipitins in a certain percent of patients. The presence of these antibodies appears to be correlated with the allergic reactions attending its administration. Further support for this rests in demonstrating positive skin tests and precipitins in man when small amounts of dextran are injected subcutaneously. Hence, until dextrans are further purified they cannot be regarded as completely inert. In addition, there is some evidence in patients with damaged kidneys that further damage occurs following infusion of moderate amounts of dextran, recovery from which may be delayed. The problems facing us with dextran are: (1) the availability of nitrogen-free (nonantigenic) material, (2) more complete control of molecular size, and (3) definition of the dose which can be given with safety to man over a given period. Dextran has been standardized for emergency use only.

f. Future Plans: Work will continue to determine the best plasma volume expander.

g. References: Progress reports of investigators listed in paragraph c.

RESEARCH AND DEVELOPMENT PROJECT CARD (NEW PROJECTS)	2. SEC. U	3. PROJ. NO. 6-64-01-009
1. PROJECT TITLE Blood and Blood Derivatives		4. REPORT DATE 30 June 52
6. BASIC FIELD OR SUBJECT Basic Medical Science	7. SUB FIELD OR SUBJECT SUB GROUP Investigations, Authorized AW-6	
8. COGNIZANT AGENCY The Surgeon General, DA	12. CONTRACTOR AND/OR LABORATORY See below	CONTRACT/W. O. NO.
9. DIRECTING AGENCY Med Res & Dev Bd, SGO		
10. REQUESTING AGENCY	13. RELATED PROJECTS	17. EST. COMPL. DATES RES. Cont. DEV. TEST OP EVAL
11. PARTICIPATION AND/OR COORDINATION	14. DATE APPROVED 5 Nov. 1951 15. PRIORITY 1-C 16.	FY 18. FISCAL EST'S. 52 278M 53 353M 54 319M
19.		

20. REQUIREMENT AND/OR JUSTIFICATION

Traumatic wounds and shock are among the most important types of casualties suffered in wartime. The treatment of these conditions almost invariably requires the use of whole blood or blood derivatives, such as plasma or albumin. Bleeding and intravascular clotting are important complications of traumatic wounds and shock, and the complications are frequently the cause of death in these cases. Many problems are inherent in blood transfusions and the administration of blood derivatives such as transfusion reactions, lower nephron nephrosis and homologous serum hepatitis. In view of the widespread use of blood and blood derivatives in the therapy of traumatic wounds and shock, it is essential that research be conducted to eliminate some of the problems arising from the administration of blood and blood derivatives and to develop ways and means of combatting hemorrhage and intravascular clotting.

21. BRIEF OF PROJECT AND OBJECTIVE

a. Brief: The objective is to study blood and blood derivatives with special emphasis on their use as therapeutic agents in traumatic wounds and shock, and to investigate ways and means of reducing or eliminating transfusion reactions and other undesirable effects of these materials. Studies will also be conducted to devise methods and techniques for fractionating, preserving and storing these materials. The basic mechanism of blood clotting will be investigated. Studies will be conducted to elucidate the factors involved in intravascular clotting and methods will be developed to promote clotting as well as to prevent intravascular thrombosis.

into its various components. These investigations are considered highly important in order to increase the available supply of these materials by separating the various constituents and thereby permitting longer storage and better preservation of whole blood. It is particularly important to increase research efforts along these lines in order to provide adequate blood and blood derivatives in the event of mass casualties from atomic warfare. Burns and radiation injury which represent a large portion of casualties from atomic explosions also require the liberal use of blood and blood derivatives to combat shock and injury to the blood-forming organs incident to ionizing radiation. Traumatic wounds represent a considerable portion of the casualties which occur from both conventional and atomic warfare, and hemorrhage and intravascular clotting are important complications of these injuries. It is therefore extremely important to investigate the basic mechanisms in the processes of blood clotting and also to devise ways and means of preventing intravascular thrombosis. Recent investigations have shown that many heretofore unrecognized factors are operative in the blood clotting mechanism and many new preparations have been advocated both to promote clotting where hemorrhage is a problem and to prevent intravascular clotting where intravascular thrombosis is a likely reaction. It is essential that these basic mechanisms be worked out and that the new preparations be investigated further in order to develop better methods for controlling hemorrhages and for preventing intravascular thrombosis. Recent efforts have also indicated that it may be possible to separate, preserve, store and later reconstitute for use, the various elements of blood such as red cells, white cells and various fractions of the plasma. These important leads must be followed and work along these lines emphasized.

b. Approach: Detailed studies will be conducted on ways and means of separating the various elements of blood and developing methods for preservation and storage, and emphasis will be placed on the development of techniques for the sterilization of plasma to inactivate the virus of homologous serum hepatitis. Investigations will also be conducted on the basic mechanism involved in the process of clotting.

The ultimate aim of this project is to provide adequate amounts of blood and blood derivatives which can be safely administered to patients requiring these materials for treatment. It is also the aim of this project to clearly elucidate the many factors involved in the blood clotting mechanism and through this understanding to develop methods for reducing hemorrhages and eliminating the common complication of intravascular thrombosis.

c. Subtasks:

(1) Massachusetts Institute of Technology - Dr. David F. Waugh

This study is designed to investigate the structure of the fibrin clot and the effect of ionic strength, pH, etc. on this clot. Attempts will

be made to determine the quantitative effect of various clot accelerators and inhibitors, and the modes of action of these substances.

(2) University of Southern California - Dr. Arnold G. Ware

This study is directed toward the isolation, purification and determination of the properties of accelerator globulin and antithromboplastin. Attempts will be made to prepare both of these clotting factors from plasma in the purest possible form.

(3) Wayne University College of Medicine - Dr. Charles L. Schneider

Will study problems related to the initiation of blood coagulation with particular reference to platelets. Special attention will be given to the platelet accelerator, the factor in platelets which influences the interaction of thrombin and fibrinogen and those activities which can be regarded as being fairly specific.

(4) Purdue University - Dr. Edwin T. Mertz

This study is directed toward the isolation and purification of profibrinolysin, fibrinolysin and anti-fibrinolysin. Attempts will also be made to determine the physical and chemical properties of these substances.

(5) State University of Iowa, College of Medicine - Dr. Willis M. Fowler

Will study blood heparin levels, plasma accelerator factors, bleeding and clotting times and platelets in animals subjected to total body irradiation, and anaphylactic shock. Similar determinations will also be made on patients with various hemorrhagic diseases and uremia.

(6) Harvard Medical School - Dr. William B. Castle

The purpose of this study is to determine by animal and human experiment *in vitro* and *in vivo* the part played by hemorrhage itself in various aspects of coagulation under normal and pathological conditions. Observations will be made *in vitro* and *in vivo* on the effect of fibrinolysis on normal and abnormal blood.

(7) Bayonne Hospital & Dispensary - Dr. Ancel U. Blaustein

(8) Harvard Medical School - Dr. Herrman L. Blumgart

Will study the "autocatalytic factors" in the pathogenesis of intravascular clotting. The purpose of this study is to obtain under controlled conditions, and using new precise analytical methods, quantitative data on autocatalytic accelerators in the in-vivo clotting mechanism. The ready availability of these accelerator substances in purified form permits this investigation.

(9) Pennsylvania Hospital - Dr. Jerome M. Waldron

Will investigate the clot accelerating properties of orally ingested fat. This investigation is concerned with the relation of fat to hemostatic mechanisms and thrombotic phenomena. In particular the anti-heparin effect of orally ingested fats will be studied in human subjects, since bleeding in irradiation injury cases is supposedly due to an increase in circulating heparin.

(10) Harvard Medical School - Dr. Benjamin Alexander

This study is aimed at the purification of prothrombin, thrombin and Acglobulin, and an investigation of their properties and interaction in isolated systems. If purification methods prove satisfactory, the chemical and physiological properties of these components will be studied using radioactive isotopes. Also antihemophitic globulin will be assayed by two-stage prothrombin consumption tests on hemophilic blood.

(11) Mercy Hospital, Baltimore, Md. - Dr. Charles E. Brambel

Will study various anticoagulant drugs such as dicumarol, tromexan and Link compound 63 (cumopyran), and special attention will be given to drugs that counteract the effects of these compounds. The counteracting drugs to be studied include oral synthetic Vitamin K, and an intravenous Vitamin K emulsion.

(12) Presbyterian Hospital, Chicago, Illinois - Dr. John H. Olwin

To evaluate the presently available methods and attempt to develop new methods for measuring known (and possibly unknown) coagulation factors of the blood, in humans, with a hope of finding some reliable means by which intravascular clotting can be anticipated. This study will be done in normal individuals, pre- and post-operative patients, undergoing major surgery and obstetrical patients during the pre- and post-partum periods.

(13) University of Virginia - Dr. Alfred Chanutin

The effect of aging of red cells on their enzymes, and other organic and inorganic constituents, and adsorptive properties will be studied. The effect of storage of plasma on the alpha and beta globulin components will be

determined. The changes in plasma proteins in the presence and absence of formed elements and the effect of the formed elements on the specific enzymes of plasma will be studied. Attempts will be made to develop procedures for preventing deterioration of red cells.

(14) University of Iowa - Dr. Elmer L. DeGowin

Blood will be collected from normal group O donors in various preservative solutions, stored for intervals from 0 to 35 days, and transfused to normal recipients of group A or B. The disappearance of donor's erythrocytes from the circulation will be measured by accurate differential agglutination techniques. The kinetics of destruction of preserved erythrocytes will be analyzed mathematically by the shape of the disappearance curves.

The respiratory rates of the preserved erythrocytes will be measured in manometers at the time of transfusion and the results compared with in-vivo survival.

Measurement of the net gain or deficit of the normal recipient's erythrocytes will be made, employing calculations based on simultaneous counts of total erythrocytes and inagglutinable cells.

In the same experiments measurements of the changes in plasma volume after transfusion will be made by the calculation of total cell volume with cell counts, hematocrits, and Ashby counts; these observations will be spot checked with estimations of cell mass by P^{32}; plasma volume will be measured by the hematocrit and cell mass and checked with the injection of T-1824.

(15) Yale University - Dr. H. H. Milstone

Will conduct studies on the isolation of prothrombin, prothrombin activators and platelets. Electrophoretic analyses will be made of the various fractions and their purified derivatives to detect unsuspected impurities. It is proposed to continue the purification of prothrombin with two objectives: 1) ultimate isolation and crystallization; 2) clarification of coagulation theory. Progress of purification will be followed by the electrophoretic and solubility tests, by comparing specific activities, and by functional tests for contamination. A lookout will be kept for a possible additional factor, distinct from prothrombin, kinase and accessory thromboplastin.

centrifugation at 4° C. Search will be made for the optimum preservative fluids for resuspending these cells to preserve them in storage. The viability, characteristics, and clinical value of the stored cells will be determined at various intervals of storage by biological tests of the infused cells. Preservation of whole blood and cell suspensions at high pressures (several hundred atmospheres) and low temperatures (-3°C to 15°C) will be assessed.

(17) Bryn Mawr Hospital - Dr. M. M. Strumia

(a) Considerable work has been done on the effect of leucocyte suspension and extract therapy on the regeneration of bone marrow of rabbits following depletion by severe total body irradiation. In previous studies an attempt had been made to purify leucocytic extracts only to find that crude preparations are more effective. An attempt to use these preparations intravenously will be made in the present course of investigation.

(b) The causes for the deterioration of the cellular elements of whole blood in storage and transport will be determined by cellular, metabolic and enzyme studies in natural and artificial substrates. Attempts will be made to slow the metabolic aging and destruction of blood cells to a minimum, by improving the preservative fluids and other means, such as dehydration, lower storage temperatures, adjustment to optimum pH, control of enzymatic activity, etc. Results will be assessed by following the viability of the preserved cells in vitro and in vivo.

(18) Harvard University - Dr. Carl W. Walter

To continue a study on preservation of human erythrocytes at -10 to -15°C in order that current blood bank storage time may be prolonged, and eventually an indefinite storage period for blood obtained.

(19) Medical Research and Development Board

It is proposed to thoroughly study the currently commercially available plastic blood containers for adaptability and suitability for military medical installations. The initial phase of this study will be to test 300 plastic blood containers from each of several commercial houses, in each of six military hospitals in Zone of Interior. The comments and recommendations from these hospitals will be combined towards the development of an ideal plastic bag for military use. The second phase of the study will include the transportation of whole blood in plastic bags to an overseas theater, for use in the military hospitals in the theater zone of communication.

The final phase will include the consideration of shipments of whole blood in plastic blood containers from the United States to an overseas theater of operations, to medical installations in the combat zone.

An additional study is to include the utilization of plastic blood containers for the collection of blood for plasma. It has been found that the use of these light-weight bags results in a considerable savings in transportation costs, and at the same time gives a 6-7% greater yield of plasma per 500cc of blood.

d. Other Information: None.

e. Background and/or Progress: Since December 1951, Walter has been searching for a better anticoagulant nutrient solution for human red cells and to determine the best technique for the long-term survival of these cells. He has developed a new anticoagulant nutrient solution containing ethylenediaminetetra-acetic acid as anticoagulant, dextrose and dibasic and monobasic phosphate as buffer. For the preservation of red blood cells, he found, that 15% glycerol prevented hemolysis following deep freezing and thawing.

Ravdin reported that the optimum temperature range for the preservation of blood to be below 4°C and above -0.5°C. The simplest procedure, according to Ravdin, for improving the preservation of erythrocytes involved the use of chilled 4.0 gm% sodium citrate, immediate chilling of blood, addition of citric acid, storage at 0°C and removal of plasma at 36 to 48 hours. The optimum pH range was 6.9-7.0. In the absence of added glucose cell deterioration accelerated after 21 days especially in concentrated preparations.

Strumia made some interesting observations on the effect of lowered temperatures of stored blood relative to bacterial contamination. He found that when Bacillus cereus (NRRL and BMH strain) were added in large quantities (i.e., 1 million organisms /ml.) to whole citrated blood and stored at 0-2°C and 2-4°C they failed to show any growth and a slight decline in numbers of bacteria was noted.

Strumia has also done considerable work on the effect of leucocytic suspensions and extract therapy on regeneration of bone marrow following depletion by severe total body irradiation. His studies indicate that rabbit plasma administered after irradiation has a protective effect on the mortality rate, independent of the leucocytosis induced by the leucocytic administration.

RESEARCH AND DEVELOPMENT PROJECT CARD (NEW PROJECTS)	2. SEC.	3. PROJ. NO. 6-59-08-014
1. PROJECT TITLE Biological and Medical Aspects of Ionizing Radiation		4. REPORT DATE 30 Jn 52
6. BASIC FIELD OR SUBJECT: Surgery	7. SUB FIELD OR SUBJECT SUB GROUP: Radiology & Roentgenology	
8. COGNIZANT AGENCY: The Surgeon General, DA	12. CONTRACTOR AND/OR LABORATORY: See below	CONTRACT/W. O. NO.
9. DIRECTING AGENCY: Med Res & Dev Bd		
10. REQUESTING AGENCY	13. RELATED PROJECTS	17. EST. COMPL. DATES: RES. Cont.
11. PARTICIPATION AND/OR COORDINATION		DEV.
		TEST
	14. DATE APPROVED	OP EVAL
	15. PRIORITY 1C / 16.	FY 18. FISCAL EST'S.: 52 334 M
19. Supersedes 6-59-08-005; 6-59-08-013		53 503 M
		54 761 M

20. REQUIREMENT AND/OR JUSTIFICATION

The atomic bomb, as a military source of ionizing radiation, requires that practical prophylactic and therapeutic measures be developed to minimize the number of casualties which may result from the use of this weapon. In addition, maximum use must be made of the beneficial features of ionizing radiation as applied to military medicine.

21. BRIEF OF PROJECT AND OBJECTIVE

a. Brief: The objective of this project is to study the basic mechanism involved in damage to living tissue resulting from ionizing radiation and to determine the prophylaxis and treatment of radiation injury. Since ionizing radiation can be used as a therapeutic or diagnostic agent relative to certain diseases which affect the body, study will be made of the advantageous application of these radiations. Likewise, ionizing radiation will be studied to investigate its utilization in therapy and in tracer and diagnostic studies.

b. Approach:

(1) Biological Effects of Radiation. Previous studies have established that radiation damage of tissue results from energy changes in the cell or its immediate environment. Recent observations suggest that the mechanism involved may include changes in the molecular composition of nuclear and cytoplasmic constituents, modification in the permeability of the cellular membrane, disturbance of endocrine function, interference with enzymatic action, and formation of toxic radicals, such as peroxides, due to the irradiation of water within cells. Microscopically observed cellular changes after irradiation include chromatin clumping or chromosome breaks, abnormal cellular division and mitosis, changes in cytoplasmic granularity, abnormal staining reactions and gross cellular changes including swelling and cytolysis. It has been noted that changes in temperature, oxygen tension, acidity and alterations in the osmotic balance of the cellular environment affect the radiosensitivity of certain tissues and biological systems.

It is planned to obtain more accurate information regarding:

22. JRDB SN.	PC.	IC & P.	I.	I.	C.

JRDB FORM 1A. 1 APR 1947

Biological and Medical Aspects of Ionizing Radiation
6-59-08-014

(a) The basic mechanism of radiation injury.

(b) Maximum single and repeated doses of radiation which may be tolerated with reasonable safety, correlated with physiological and clinical effects.

(c) Hazardous doses which may cause some temporary or permanent damage, including some casualties and possibly some deaths, but which are acceptable as military risks.

(d) Casualty-producing doses which should lead to evacuation from contaminated areas whenever possible.

(e) Toxicity of inhaled and ingested radioactive materials.

(f) Effects of irradiation as modified by other complications such as thermal radiation, war wounds, infectious agents, and individual and group susceptibility.

(g) Extent and nature of injury by ionizing radiation from electromagnetic radiations of different wave lengths and energies and from different corpuscular radiations of varying energies.

(2) Prophylaxis and Treatment of Radiation. Efforts are being intensified to identify a biological factor which will promote recovery from radiation injury. It has been established that anoxia prior to irradiation and maintained during the irradiation period increases the survival rate. It has been found that compounds such as para-aminopropiophenone (PAPP) methemoglobinemia is effective by producing a decrease in tissue oxygen. Likewise, such compounds as pitressin, epinephrine, and serotonin by vasoconstrictor action and resultant anoxia provide protection. Many sulfhydryl compounds, such as cysteine, glutathione, and BAL have been found to increase the survival rate. Partial body-shielding has also been effective. In particular, significant increase in the survival rate in animals has been obtained by shielding the spleen, abdomen, adrenals, extremities, liver, lungs, and head. A practical military application of these findings has not been accomplished.

Post-irradiation, it has been found that antibiotics of various types administered to different experimental animals increase both the survival time and rate. Transplantation of the spleen has increased survival. Cross circulation in dogs and parabiosis in rats accomplished after irradiation appear to influence radiation

(3) Utilization of Ionizing Radiation in Therapy and in Tracer and Diagnostic Studies. Radioisotopes are now being employed to supplement and occasionally replace x-ray and radium in the treatment of cancer and allied diseases. They may be used externally or internally. Studies will be made of therapeutic value and diagnostic possibilities of different radioisotopes and sources of ionizing radiation.

Because of the extreme sensitivity of detection and the unique specificity of radioisotopes as "tags" they are of tremendous value in identifying certain types of cells. They permit the study of countless processes going on in the body which otherwise would be obscure. Radioisotopes will be used to investigate these fundamental processes and thereby assist in the solution of complex research problems.

c. Subtasks:

(1) Army Medical Service Graduate School

(a) To study the value of prophylactic and chemotherapeutic agents and various protective measures which will alter or minimize injury and mortality from whole-body or partial-body irradiation.

(b) To study the utilization of radioisotopes in tracer and diagnostic procedures.

(2) Army Medical Research Laboratory

(a) Effects of Ionizing Radiation. The early effects of x-irradiation damage are being studied at the cellular level by means of various histochemical and biochemical techniques.

(b) Enzyme, Endocrine and Metabolism Studies in Total-Body Irradiation. The alterations in the activity of various enzyme systems and in the function of various endocrine organs of animals receiving total-body x-irradiation are being studied in an attempt to determine effective pharmacological and chemical means of preventing the damaging effects of ionizing radiations.

(3) University of Chicago - Dr. J. Garrott Allen

Ionization Effects. To study the effect of ionization on experimental animals and human subjects; specifically, local injuries incident to irradiation, fluid balance, hemorrhage and infection following irradiation with x-rays. Observations will include pathological, physiological and biochemical factors.

(4) Tulane University School of Medicine - Dr. G. E. Burch.

Potassium metabolism as determined by radioactive rubidium (Rb^{86}). These studies will aid not only in the understanding of rubidium metabolism and rates of turnover, but, when correlated with the simultaneous measurement of ordinary potassium, will also indicate the accuracy with which radiorubidium traces potassium in the body of man. These studies will be of value in understanding the effects

of atomic radiation on the human body.

(5) National Academy of Sciences - Dr. Webb Haymaker

To conduct a pathological study of the central nervous system of irradiated humans and experimental animals.

(6) Ohio State University Research Foundation - Dr. Milton A. Lessler

To study the effects of low-level radiation on the nucleus, cytoplasm and metabolism of nucleated erythrocytes.

(7) University of Pennsylvania - Dr. E. P. Pendergrass

To study the biological effects of atomic irradiation in man and animals. These studies will include the investigation on methods of action of radiation on mammalian tissues with particular reference to protection from the damaging effects of atomic irradiation. The effects of a variety of anoxic and pharmacologic agents which alter radiation sensitivity will be studied. Similar human studies will be carried out for correlation of the protective effects of the same drugs and agents.

(8) Cedars of Lebanon Hospital - Dr. Peter F. Salisbury

Dogs will be exposed to standard lethal dose of radiation. The test animals will then be cross-transfused with normal dogs. The following factors will be varied: time interval between irradiation and cross transfusion, rate of blood, amount of blood exchanged and age of animals.

d. Other Information: None.

e. Background and/or Progress: At the Army Medical Service Graduate School it has been shown that dogs receiving aureomycin had a lower mortality rate than untreated animals. It has been found that prepubertal orchiectomy was markedly protective to mice irradiated during the pubertal or postpubertal period. The mortality rate following irradiation of puberally castrated mice was restored to that of intact males by administration of testosterone.

The Army Medical Research Laboratory found that pre-irradiation intraperitoneal injections of serotonin in rats resulted in a survival rate of 97%. Pretreatment with para-aminopropiophenone and sodium [illegible]

The Laboratory also reports that intraperitoneal injections of whole baby rat spleen homogenates and whole baby mouse homogenates showed some protection from irradiation.

Allen reports that the frequent administration of fresh blood transfusions failed to improve the survival rate or to ameliorate spontaneous bleeding after whole-body x-irradiation. On the basis of his experiments, it appears that a more cautious attitude must be adopted toward the use of frequent blood transfusion alone as a therapeutic measure in the treatment of the latent symptoms of irradiation injury in man. Allen has found that dogs whose heads had been shielded were greatly protected when the remainder of the body was exposed to 450 r x-radiation.

Salisbury has been studying the effect of cross transfusion between normal animals and animals exposed to lethal doses of x-radiation. His preliminary studies indicate that irradiation injury could be altered by a single cross transfusion administered post-irradiation, indicating the existence of some protective substance or mechanism.

f. Future Plans: Studies will continue.

g. References: Progress reports of investigators.

Project No. 6-59-08-14
Contract No. MD-93

NOT FOR PUBLICATION
Report Date 30 June 1952

Ionization Effects

Dr. J. Garrott Allen
University of Chicago
Chicago, Ill.

BRIEF. The purpose of this work is to study injuries incident to irradiation, the physiologic and biologic effects following suhh irradiation, and the development of therapeutic methods to combat irradiation injuries.

BACKGROUND. This contract was effective 1 November 1947. The report of December 1950 gave details of findings on irradiated animals up to that time (exercise tolerance, nutrition, anoxia, infection, etc.). All efforts to establish that blood transfusions are advantageous in the treatment of radiation sickness in dogs were negative. Differences among laboratories regarding roentgen dosages as they relate to mortality in dogs made it seem that the only basis for comparison was the survival rate at a given dose; i.e., comparable survival figures for dogs in the various laboratories rather than on equal roentgen dosages in each laboratory. From work on the role of infection in radiation injury it was concluded that septicemia caused by micro-organisms of enteric origin was largely responsible for the death of mice irradiated with the doses of x-ray used (1A report, 30 June 1951). A survival of 18% was found in animals treated with whole-blood transfusions and aureomycin. The latest information on blood transfusions and on the protective effect of head shields appears under PROGRESS, below.

PROGRESS. (1 Nov. 1951 - 30 June 1952). 1. Protective Value of Lead Shields. As a result of studies on 30 dogs it was found that: (a) Dogs whose heads had been shielded by 1/16" or 1/32" of lead were greatly protected when the remainder of their body was exposed to 450 r x-irradiation. This same dose, when the head is not protected and the animal is given no medication, is uniformly fatal here. Stated otherwise, a shield permitting not more than 50 r penetration to the head and allowing 450 r exposure to the remainder of the animal, enabled 90% of the animals to recover, whereas all those receiving no head shielding died. (b) The animals which died despite head shielding exhibited the findings characteristic of the unprotected irradiated dogs receiving 450 r.

2. Blood Transfusion in Irradiation Hemorrhage. Among the postirradiation findings characteristic of near-lethal (LD_{50}-LD_{100}) exposures to ionizing radiation are spontaneous abnormal bleeding and anemia. Because of the prominence of thrombocytopenia and anemia in the abnormal bleeding syndrome of irradiation sickness, it is natural to assume that the frequent administration of fresh whole-blood transfusions might be of considerable therapeutic value in the control or prevention of this type

This is a study of the therapeutic value of blood transfusion given, without antibiotics, to determine whether this procedure will prevent irradiation hemorrhage and/or improve the survival rate in the x-irradiated dog. The dog was chosen because in many respects its response to total-body irradiation is similar to man's. This animal differs in 1 important respect in that its blood types are much less well defined. The results obtained from transfusion alone were the reverse of those anticipated.

One hundred and seventy-three dogs were exposed to single doses of total-body x-irradiation at the following dosage levels: 175, 225, 275, 325, 375, and 450 r. The animals were divided into 2 groups; one group of 101 dogs served as controls, and the other group of 72 was transfused with citrated fresh whole blood 3 times a week beginning on the 4th postirradiation day. Five ml/kg of body weight was administered on each day the animal was transfused. In addition to this blood the animal received a volume of blood equivalent to the amount withdrawn for study just prior to each transfusion. No other treatment was administered.

From this study it was concluded that the frequent administration of fresh blood transfusions without antibiotics in dogs failed to improve the survival rate or to ameliorate spontaneous bleeding after exposures to total body x-radiation, (LD_{50}-LD_{100}). On the basis of these experiments a more cautious attitude toward the use of frequent blood transfusion alone as a therapeutic measure in the treatment of the latent symptoms of irradiation injury in man may be indicated. These data do not relate in any manner to the use of blood in the treatment of shock incident to the early blast effects of an atomic burst, or to blood needs in anoxic anemia, where the therapeutic importance of adequate blood and plasma transfusion is soundly established.

3. Blood Transfusions and Aureomycin. More recent studies on 53 irradiated dogs indicated that: (a) Blood transfusions given alone 3 times a week are of no demonstrated benefit in dogs exposed to 375 and 450 r. Animals receiving aureomycin alone also showed no benefit at 375 r or 450 r. The possible exception of a minimal benefit of whole blood transfusions combined with aureomycin therapy still persists. (b) These data are not to be compared with the reports of Salisbury et al., who obtained some benefit when complete exchange transfusions were performed within a few hours after irradiation exposure.

4. Experience with Transfusions of Platelet Concentrates in Dogs Receiving 450 r Total Body Irradiation. Platelets were collected by exsanguinating donor dogs 4 days after a turpentine abscess was produced. Silicone technique was used throughout and sequestriene was used as the anticoagulant. By sedimentation and differential centrifugation it was possible to recover approximately 50% of the platelet from the donor dogs. These were prepared daily and administered as daily transfusions to 7 irradiated dogs exposed to 450 r x-radiation. The recipients were given the platelet concentrates intravenously beginning between the 5th and 7th days and continuing until the animals' deaths. The number of platelets given were sufficient to raise the platelet count from its thrombocytopenic level to near normal concentrations. In spite of this all animals died, as did their controls. There was no increase in survival time. The whole-blood clotting time was reduced to some extent following platelet transfusions but not returned to normal. When an animal received sufficient platelets to return his platelet count to a normal level, the number of platelets rapidly disappeared and in 24 to 36 hours the pre-existing profound thrombocytopenia was again present.

From this study it may be concluded that (a) Platelet cnncentrates can be given in sufficient quantities to return the platelet count of the irradiated dog from his thrombocytopenic levels to normal levels. (b) The entire blood volume of at least 2 animals a day for each day each irradiated animal was transfused was required to obtain a sufficient number of platelets. In the case of the animal receiving 10 platelet transfusions, more than 20 donor animals were sacrificed for this experiment. (c) A dreduction in the extent of hemorrhage was obtained by platelet transfusions but this in turn had no effect upon the morbadity either in terms of rate or in terms of survival.

5. Summary of Irradiation Studies to Date. (a) Does head shielding of the head of dogs by lead and then exposure to 450 r increase the survival rate and time? Yes. This is the most striking phenomenon and may form the basis upon which some protection from irradiation injury may be planned. (b) Is the frequent administration of whole-blood transfusions beneficial in extending the life of the dog or in reducing the mortality from total body irradiation? No. (c) Is aureomycin alone and given at intervals twice a day commencing with the day of irradiation beneficial in extending the life of reducing the mortality rate in the 450 r and 375 r exposed dogs? There was no evidence of a reduction in mortality rate although there was some evidence of a slightly increased survival time. (d) Is aureomycin combined with whole-blood transfusions beneficial in reducing the mortality rate or in prolonging the survival time in the 450 r or 375 r exposed dog? Three of 21 animals so treated survived receipt of whole-blood transfusion 3 times a week and aureomycin twice a day following irradiation. At 450 r and without any treatment there are no survivors in several hundred controls. (e) Will platelet concentrates given in quantities sufficient to maintain the platelet count within near normal range influence the survival rate or the survival period in the 450 r dog? The answer seems to be no, although only 7 animals have been studied. Some reduction in hemorrhage was observed, but this had no influence on the survival time. (f) Are transfusions given 3 times a week beneficial when administered to animals which have received lower levels of exposure? The answer is no.

6. Homologous Serum Jaundice and Its Relation to Methods of Plasma Storage. Observations over a number of years (at University of Chicago Clinics) regarding the danger of hepatitis from transfused stored plasma have shown that: (a) Lyophilization, freezing, and refrigeration, almost exclusively employed in the preservation of plasma, are also the most suitable means available for the preservation of virus activity. As long as these methods are employed for the storage of plasma, it is imperative that pooled plasma be sterilized in some manner before such storage procedures are instituted in order to reduce or abviate the troublesome incidence of homologous serum jaundice from pooled plasma.

(b) No virus is known to survive prolonged room temperature in a cell-free liquid state without preservatives. In the University of Chicago Clinics, where plasma has been stored at high room temperature for a period of 3 to 12 months before use, it appears doubtful that any patient has contracted homologous serum jaundice from this plasma. Of 864 patients here given units of pooled plasma stored in the liquid state at 78 to 98°F. for 3 months or longer, 3 are known to have contracted homologous serum jaundice, The source of infection in these patients seemed more likely due to the blood received by 2 of them or to the lyophilized plasma or blood given the 3rd patient. None of the 212 patients here who received plasma alone was known to have had latent jaundice. This plasma appears undiminished in its nutritive value and is remarkably free of elements causing untoward reactions.

(c) During the same period, at least 14 and probably 16 or 17 of 6,503 patients receiving only refrigerated whole blood transfusions manifested latent jaundice typical of the homologous serum jaundice syndrome.

REFERENCES.

Allen, et al., (Irradiation): Science, May 9, 1952, Vol. 115, No 2993, p. 523-526.

Allen, J. G., Moulder, P. V., and Enerson, D. M. J., (Irradiation) J. Am. Med. Assoc., 145, 704 (1951).

Allen et al., (Plasma and Hepatitis): J. Am. Med. Assoc., November 25, 1950, Vol. 144, pp. 1069-1074.

Project No. 6-59-08-14
Contract No. MD-182

NOT FOR PUBLICATION
Report Date 30 June 1952

Radioactive Tracer Studies

Dr. G. E. Burch
Dept. of Medicine
Tulane University
New Orleans, La.

BRIEF. The objective is to study potassium metabolism as described by radioactive rubidium (Rb^{86}).

BACKGROUND. This contract was effective 1 June 1951. Some pertinent work under an earlier contract was summarized here in December 1951 under 6-64-12-12.

Turnover studies of potassium metabolism by means of Rb^{86} began last August in 2 control human subjects and 2 subjects with chronic congestive heart failure. Immediately after intravenous injection of the radiorubidium, rapid collections of blood and urine were made so that the immediate concentration-time course in red blood cells, plasma, and urine could be studied. Collections were made daily of all excreta passed and of blood for about 6 weeks. One additional control and 2 more chronic congestive heart failure cases were treated by the same procedures when a new shipment of Rb^{86} arrived and followed for 60 days. Analysis of data appears under PROGRESS.

Injections of Rb^{86} and K^{42} were made simultaneously by means of the same syringe; then samples of blood and urine were collected at a rapid rate for the 1st 2 hours and than at longer intervals until the K^{42} decayed to radioactive levels too low to permit accurate counting. The subjects were studied for about 8 weeks until the Rb^{86} reached levels in the urine and blood which were near background. Ordinary Cl, Na, and K were measured in their blood and urine.

PROGRESS. These experiments were aimed at comparing metabolism of potassium and the related elements, rubidium and cesium, in an isolated system composed of human cells. Human whole blood was maintained under simulated physiologic conditions in-vitro, and the isotopes, K^{42}, Rb^{86}, and Cs^{134}, traced the movements of the respective elements. It was possible to measure the partition of these elements between the erythrocytes and plasma, the speed of their transfer across red cell membranes, and to some extent the mechanisms of this transfer into and out of cells. Red cells metabolized potassium and rubidium in identical fashion, within the limits of measurement. Cesium, on the other hand, although qualitatively similar to potassium and rubidium, showed important quantitative differences.

Other workers have shown that a 10^{o} C rise in insulation temperature will more than double the potassium exchange rate (K^{42}) of red cells. Since changes in temperature of this magnitude produce much smaller changes on strictly physical processes such as diffusion across membranes, this finding indicates that chemical reactions are involved in the transfer of potassium into and out of the red cells. These experiments showed that a similar process is involved in the transfer of rubidium and cesium.

These findings indicate that K^{42}, Rb^{86}, and Cs^{134} are metabolized similarly by human red cells. Since no difference was detected between rubidium and potassium in this particular human cell, it is suggested that under some circumstances Rb^{86} ($T_{\frac{1}{2}}$ 19.5 days) might be used satisfactorily to give qualitative and semiquantitative information about potassium metabolism under conditions where K^{42} is unsuitable because of its 12.4-hour half-life.

Partial analysis of the in-vivo experiments using K^{42} and Rb^{86} simultaneously in man suggests that although rubidium and potassium appear to be metabolized in a similar manner by the red blood cell, there are differences in the specific activities of these 2 elements in other areas, namely, the urine and blood plasma in the intact man.

Analysis of data from studies using Cl^{36} in humans indicated that there exist discrepancies between the chloride space determined by the isotope dilution method and those which may "actually" exist. These differences are conceivably due to the sequestration of tracer in compartments which are not fully exchanging with the sampled compartment. The discrepancies in space determination lead to similar discrepancies in the determination of the "total" body electrolyte by the same method. The numerous variations which may occur among the compartments of the tracer, non-tracer, and water have been described.

FUTURE. Studies on red cells are continuing, using Cl^{36} to compare chloride behavior with Rb^{36}, K^{42}, and Na^{22}.

REFERENCES: Burch, G. E. et al., "Estimation of the Time of Equilibrium of Distribution of Long-life Radiochloride and Radiosodium in Man with and without Congestive Heart Failure," Acta Med. Scand., 142:329-341, 1952.

Project No. 6-59-08-14
Contract No. MD-263

NOT FOR PUBLICATION
Report Date 30 June 1952

Analysis of Indirect Effect of Radiation

Dr. Peter F. Salisbury
Cedars of Lebanon Hospital
Los Angeles, California

BRIEF. The aim of this study is to determine the effect of cross-transfusion between normal animals and animals exposed to lethal doses of irradiation.

BACKGROUND. This contract was effective 1 February 1952. The results reported appear under PROGRESS.

PROGRESS. Preliminary studies in this laboratory indicated that irradiation injury, as measured by the clinical condition, the hemogram and mortality, could be altered by a single cross-transfusion procedure administered after irradiation.

Control Studies. Twenty-five selected adult mongrels exposed to 450r total body irradiation showed a mortality of 95% in a 30-day period.

Cross-Transfusion Experiments. Twenty-two dogs cross-transfused within 4 hours after irradiation showed a mortality of 27%, associated with improvement in the clinical state of the animals (increased physical activity, better food consumption), improvement in the hematologic picture, and a conspicuous reduction of hemorrhagic and infectious manifestations. The results are further evidence of the existence of a protective effect of cross-transfusion in radiation disease.

Studies of the Nature of the Protective Substance. In order to determine the nature of the substance (s) responsible for the protective effect it is necessary to determine whether this effect is linked to the transfer of cellular elements which occurs during a cross-transfusion procedure. In order to separate cellular from humoral factors, cross-dialysis - the dialysis of blood against blood - is considered a logical experimental procedure. If the protective effect is found not only after cross-transfusion but also after cross-dialysis it would then be assumed that the protective principle is a molecule which can diffuse through a cellophane membrane. Such a finding would be all the more important because it would make it easier to isolate and identify the protective substance. Blood, as distinct from the spleen or the bone marrow of mice, is available in large quantities as a raw material from which trace molecules may be isolated.

When 6 cross-dialyses were performed on irradiated dogs a suggestive protective effect was observed in some instances. Infusions of dialysate from normal dogs into irradiated animals were also done; the results were inconclusive and not statistically significant. It was decided that, before further experiments are performed, a more effective dialyser must be obtained and the available equipment perfected.

The need for a more effective dialyser is explained by theoretical considerations. The protective substance may well be a relatively large molecular weight. A large molecule will migrate through a cellophane membrane at a very small rate. If such a hypothetical molecule is to be transferred from the blood of a donor, where it is present

in minute amounts, to the blood of the test animal, where it may have been destroyed by ionizing radiation, very prolonged cntact of the blood with a very large membrane area will be required for the molecule to be transmitted in sufficient amounts; hence the need for a dialyser with a very large cellophane area and other special characteristics. A device now available is considered very adequate for cross-dialysis; this machine has a maximum cellophane surface of 42,000 sq cm and a maximum blood flow of 400 cc per minute.

Hemorrhagic phenomena are responsible for a large proportion of the morbidity and mortality of irradiated mammals. At present cross-dialysis can be performed only in heparinized animals. It seems particularly inappropriate to treat irradiated animals with anticoagulants, because this may increase the incidence and severity of hemorrhagic phenomena due to radiation disease. Therefore, it would seem desirable to invent and develop means of cross-dialysis which would obviate heparinization of th the experimental animal. Equipment was made to heparinize blood as it is withdrawn from a dog in a cannula, and to neutralize the heparin in the withdrawn blood before this is returned to the animal body. This equipment, still in the developmental stages, is ready for initial testing.

Cross-dialysis was developed to exclude the role of formed elements in the causation of protective effect. In a multiple-layer type dialyser occasional leaks occur between the 2 compartments, which transfer small numbers of cells between the compartments and thereby vitiate the experiment. These leaks are detected easily when 1 compartment is filled with blood and the other with dialyzing solution, but not when both compartments are filled with blood. A method for the instantaneous detection of small leaks between the 2 compartments is needed. A tracer substance such as Evans Blue (T 1814) can be injected into the donor animal, and a monitoring device, such as a photoelectric unit sensitive to blue light, will detect the dye in the blood circuit of the irradiated animal. The design and availability of this equipment have been considered.

Cross-transfusion exerts a protective effect, yet there is a certain mortality in irradiated dogs treated with this procedure. Cross-transfusion with the available blood pumps is considered to destroy a small percentage of the platelets and leukocytes in the blood. Would an improved cross-transfusion pump, which does not need heparin and which does not destroy formed elements, improve the mortality ratio? In order to answer this question it is necessary to have improved equipment for cross-transfusion and for blood pumping in general. In such equipment now under construction blood comes in contact only with plastic material. The blood circuits will be shortened by about 70%, the pumping action will be gentle and smooth, and the measurement of the transferred volumes will be as precise as in the present machine. It is expected that the new cross-transfusion device will not only be non-hemolytic but also will not destroy significant numbers of platelets ar leukocytes.

FUTURE. Before the new apparatus can be used in cross-dialysis it must be tested for mechanical performance, clearance, and for its influence on the blood pressures of normal dogs whose blood will be passed through the 2 compartments. It will be ready for use only when it has been found free from undesirable side effects, such as hypotensive or febrile reactions. When the procedure for cross-dailysis is developed and standardized, the device for extracorporeal heparinization will be used in cnnjunction with it. Improved cross-transfusion devices will be used as soon as they are ready.

Project No. 6-59-08-14

NOT FOR PUBLICATION
Report Date 30 June 1952

Effects of Irradiation

Army Medical Research Laboratory
Fort Knox, Kentucky

Early Effects of Ionizing Radiation

BRIEF. The aim of this work is to study the 1st effects of x-irradiation damage at the cellular level by various histochemical and vital-staining techniques, and to correlate these results with findings on total-body irradiation.

BACKGROUND AND PROGRESS. Protection. Cysteine has been shown to influence the effects of x-rays on nucleic-acid metabolism. The inhibiting effect of x-rays on nucleic-acid formation is restricted to some degree in the cysteine-treated animals.

The possible protective effects of ferritin and reticulose are being studied.

Cobalt-protection studies were continued, with particular attention given to methods of administration, influence of time intervals, etc.

Tetrazolium salts, which protect when given before irradiation, were further investigated to determine whether any specific vital organ was involved. Liver studies have so far shown no alteration in enzymic activity.

The implantation of whole rat spleen into rats after irradiation afforded no protection but the intraperitoneal injection of whole baby rat homogenate into rats after being irradiated (880r) afforded some protection as judged by survival times. Whole mouse embryo homogenate gave no protection but whole baby mouse homogenate showed some little protection.

Effect of Irradiation on Mitotic Activity. The effects of soft and hard x-rays on the structural changes in the cornea of the rat were studied. The effects on mitosis previously reported were not altered by using rats of different ages or strains nor by shielding various parts of the body. BAL afforded no protection, while cysteine gave slight protection.

Combination of Ionizing Radiation and Thermal Burns. Mice treated with combinations of thermal burns and x-irradiation died earlier and in greater numbers than those receiving x-irradiation only.

FUTURE. Experiments with cobalt will be continued and extended. Ferritin and reticulose studies will be done in combination with blood studies in an attempt to shed light on the question of whether a humoral or a cellular factor is responsible for the effects noted with tissue homogenates. The tetrazolies will be studied, if possible, microchemically on a cellular level.

The early effects on the mitotic changes in the rat cornea will be correlated with dosage levels and various chemical protecting agents.

The method of Pelc (radioautographs of single cells with P_{32}) will be applied to special irradiation problems on cells, to determine the interrelation of chemical and morphologic changes; i.e., the Feulgen reaction.

Studies on long-period, low-intensity, total-body irradiation were begun and will continue for 12 or more months.

Effect of Irradiation on Single Cell Organisms

BRIEF. Studies are being made on unicellular organisms in order to obtain an uncomplicated picture of cell damage and possible adaptation to irradiation and other stresses.

BACKGROUND AND PROGRESS. Extension of investigations on the effect of irradiation on single-celled organisms led to the following observations.

Heavy doses of ultraviolet light caused a stimulation of the endogenous metabolism, as measured by the rate of methylene blue reduction, in yeast cells (Saccharomyces cerevisiae). There are indications that the extent of this stimulation is a function not only of cell age but also of the carbon source used for cell growth. The ability to stimulate endogenous metabolism has been shown not to be confined to ultraviolet light alone. Heavy doses of soft x-rays (250,000 r) on intact yeast cells, far from inhibiting methylene blue reduction, actually produced a manifold stimulation of the endogenous metabolism.

Ultraviolet irradiation of an isolated crystalline enzyme, alcohol dehydrogenase, containing oxidizable sulfhydryl groups, resulted in a rapid inactivation. Irradiation of the enzyme with visible light after exposure to ultraviolet resulted in further inactivation. In contrast to the purified alcohol dehydrogenase, this enzyme when in the intact yeast cell suffered no significant loss of activity after irradiation with ultraviolet light.

FUTURE. An investigation into the nature of the endogenous material which is being oxidized after ultraviolet and x-irradiation is contemplated.

Enzyme, Endocrine, and Metabolism in Total-Body Irradiation

BRIEF. The alterations in the activity of various enzyme systems and in the function of various endocrine organs of animals receiving total-body x-irradiation are being studied to determine effective pharmacologic and chemical means of preventing the damaging effects of ionizing radiations.

BACKGROUND. Studies on the biochemical changes in various organs and tissues produced by total-body x-irradiation were reported in the last 3 issues.

Correlation between Tissue Oxygen Tension and Radiation Sickness.

PROGRESS. Rats were subjected to a dosage of 880 r, giving a 28-day survival rate of 10-14%. Serotonin (5-hydroxytryptamine), at a dose level of 4 mg/kg body weight injected 5 minutes before exposure, produced little if any protection, but 20 mg/kg given at the same interval prior to exposure produced a striking protective effect, as indicated by a survival rate of 97%.

Pre-treatment with para-aminopropiophenone in doses of 32 and 16 mg/kg 30 minutes

before x-ray exposure, producing methemoglobin levels of 70 to 78%, resulted in 28-day survival rates of 94 and 97%, respectively. Pre-treatment with para-amino-propiophenone at a dose level of 6 mg/kg or with sodium nitrite 60 mg/kg, producing methemoglobin levels of 52 to 56%, resulted in 28-day survival rates of 47 and 30%, respectively.

The protective effect elicited by serotonin (20 mg/kg) is assumed to result from the vasoconstrictor property of this agent, causing a transient tissue anoxia in a manner similar to that of epinephrine (Gray, et al., Proc. Soc. Exp. Biol. & Med., 79: 384, 1952).

The protective effect of methemoglobinemia at certain concentrations probably is caused by a decreased supply of oxygen to the tissues which renders them anoxic.

The findings on the protective action of serotonin and para-aminopropiophenone against radiation seem to substantiate the concept of a relationship between tissue oxygen tension and radiosensitivity.

FUTURE. This subtask will be terminated as such and studies on protection continued under the 1st subtask, Early Effects of Ionizing Radiation.

REFERENCE. "Protective Effect of Pitressin and of Epinephrine Against Total Body X-Irradiation," Proc. Soc. Expt'l Biol. & Med., 79, 384, 1952.(taken from AMRL Report No. 64, 10 Sept., 1951).

Quantitative Studies on the Effects of Non-Ionizing Radiation on the Skin

BRIEF. Quantitative studies of the spectral reflectance of the skin are being made in order to evaluate the effects of ultraviolet radiation in producing erythemas and tans, and to determine the ultraviolet absorption by the skin.

BACKGROUND AND PROGRESS. Summaries on the spectral reflectance of white and Negro skin between 440 and 1000 mμ and on the absorption of human skin between 440 and 1000 mμ for black body radiation at various color temperatures were given in June 1951. The effects of ultraviolet radiation on certain amino acids were reported last quarter.

Collection of additional reflectance data on white and Negro skin (male and female), in order to establish a uniform statistical basis for the 2 skin colors and the 2 sexes, will be continued in September in order to obtain data comparable to those already collected with regard to the seasonal factor.

white rats will develop a blister with resulting scar formation. The minimum energy necessary to produce these effects at 300 mμ was determined. Other wave lengths were tried.

FUTURE. Theoretical study will be undertaken to determine whether a method developed in 1931 by Kubelka and Munk can be applied to establish a quantitative relation between the concentration of the pigment and the resulting skin color. More data in the 1,000 to 1,360 mμ range will be collected to extend the knowledge of the reflection and absorption properties of the skin under varied pigmentations.

Irradiation experiments with known amounts of energy and measurements of the resulting skin color changes and of their time course will be begun. Studies on melanin will be continued. Animal experiments will be made to investigate factors such as blood circulation, cold, heat, etc., which influence the UV erythema and tan

REFERENCE. "Spectral Reflectance of White and Negro Skin Between 440 and 1000 mμ," J. Applied Physiol., 4;800, 1952 (taken from AMRL Report 6-64-12-08-(2), 1 May 1951).

Biophysical Study of Burns

BRIEF. Spectral reflectance studies are being extended to include the near ultraviolet and the infrared radiations in order to evaluate time-intensity relations to the degree of skin damage.

BACKGROUND AND PROGRESS. A semi-quantitative reflectance study was made of animal skin before and for 3 to 5 days following exposure to thermal energy, by means of the G.E. recording spectrophotometer.

The production of an erythema, with a resulting blanching of the skin surface, involves an increase of the reflectance values throughout the range 400 to 700 mμ; however, when charring of the skin surface occurs a decrease in the skin reflectance values results. An increase in the exposure temperature from 230 to 300° C. has a greater influence on the color of normal guinea pig and rabbit skin than does an increase in the time of exposure from 5 to 10 seconds. At temperatures of 230° C. and above, with exposure time of from 5 to 10 seconds, a charring of the skin surface results. The use of depilated animals was found to be justified since no difference could be found spectrophotometrically in the skin colors of furry and hairless mice.

FUTURE. The instrumentation will be perfected according to experience gathere from the Naval Radiological Defense Laboratory, San Francisco, and then recalibrated with the thermocouple recently calibrated at the National Bureau of Standards. An improved shutter is under construction. Heat erythema and burn experiments under controlled conditions will be undertaken.

Project No. 6-59-08-14

NOT FOR PUBLICATION
Report Date 30 June 1952

Ionization Effects

Army Med Sv Grad School
Walter Reed Army Medical Center
Washington, D. C.

BRIEF. The objective in the 1st 2 groups of tests described is to explore diagnostic and therapeutic uses of radioisotopes.

I. I^{131}

BACKGROUND AND PROGRESS. Clinical Use of I^{131}. From 1 April to 20 June 1952 144 patients have been studied using 1 or more of a battery of diagnostic procedures following the oral administration of a tracer dose of radioiodine.

Three-day Slopes. Serial determinations of I^{131} uptake have been made during the past year in 175 patients, observations having been made at 24, 48 and 72 hours following administration. This was undertaken in order to assess the rapidity of release of newly formed thyroid hormone in a variety of chemical conditions. In thyroidal states in which release of newly formed hormone is accelerated, it is to be expected that the curve of I^{131} uptake will turn downwards at a relatively early interval. Where release is slow the time at which the curve turns downward should be delayed. Normal thyroid function should show curves which lie intermediate between these 2 extremes. Measurement on 3 successive days permits definition of the time interval required for release of radioactive hormone from the thyroid gland to exceed new formation, of radioactive hormone. Analysis of the data reveals that in the patients in whom the release of thyroid hormone would empirically be expected to be accelerated the values at 24, 48, and 72 hours show a progressive decline. In normals the pattern has been 1 of either a continuous increase during the 3-day period or an increase in the 1st 2 days with a turn downwards in the 3rd day. The possibility that the differences between these 2 types of normal curves may represent normal response of the thyroid gland to changes in environmental temperature is being investigated.

serial study of thyroid hormone metabolism in single patients. It also made it possible to investigate the state in which thyroid hormone is transported in the blood. Ultracentrifugal studies have been carried out, as well as studies of the radioactive hormone content of various serum protein fractions. These studies reveal that the distribution of iodine bearing moieties between the protein fractions of the serum is nonspecific.

Time-Concentration Curves. Radioactive thyroxine determinations have been carried out for a prolonged period following administration of therapeutic amounts of this isotope in 8 patients suffering from hyperthyroidism or thyroidal carcinoma. Two distinct types of curves have been noted. The patients receiving massive doses of radioactive iodine have displayed a progressive rise in the serum concentration of radioactive hormone for about 2 weeks following administration then a very sharp and sudden increase. The peak of this increase was followed by a fall in the serum concentration of radiothyroxine, producing an exponential type of decline. It is believed that the sudden rapid rise in radiothyroxine concentration represente the temporary or permanent destruction of the thyroid follicle with release of store radioactive hormone and the subsequent exponential fall represented the time exponer ial disappearance rate of this released radioactive hormone, under conditions in whi a physiologic damage to thyroid tissue prevented other further reduction and release of tagged hormone. The 2nd pattern has been observed in patients receiving small dc of radioactive iodine, and consists of a gradual rise in serum concentration of radi active hormone followed by a plateau of varying duration and a gradual decline. Thi pattern is believed to be the result of continued although diminishing function of thyroid gland superimposed upon the utilization rate of radioactive hormone from the blood.

Thyroiditis. During the past year 3 patients with the rare disease of acute c subacute thyroiditis have been studied, utilizing the modalities developed in this l oratory. This disease presents a puzzling picture in that patients offer no clinica evidence of hypothyroidism and in fact may clinically and by laboratory tests be mi hyperthyroid and yet take up little or no radioactive iodine. Accumulation gradient performed in these 3 patients reveal that the thyroid is indeed capable of accumula radioactive iodine, but not of holding the accumulated iodine within the gland, so by 24 hours the accumulated iodine has virtually all left the gland. Twenty-four-h uptake is therefore subnormal. Simultaneous measurements of the serum radioactive mone reveal that radioactive hormone is being formed but is not being stored within gland. It would therefore appear that the physiologic abnormality in this disease sults from inability of the thyroid gland to retain thyroid hormone. This theory c related well with the histologic picture of thyroiditis in which dissolution of fol lar walls and phagocytosis of extrafollicular colloid is seen.

Three cases of toxoid hyperthyroidism have been treated with radioactive iodi One case of adenocarcinoma of the thyroid has been treated with a therapeutic dose.

The Chemical Nature of the Thyroidal "Iodide Trap". The thyroid gland is dis guished by its unusual and as-yet-unexplained capacity to concentrate iodide ion fr the serum and achieve an interthyroidal concentration of iodide which often exceeds the serum iodide concentration. This iodide has been demonstrated present in a for which is freely dialyzable and potentiometrically indistinguishable from iodide ion The chemical mechanism underlying this remarkable concentrating capacity is virtual unknown. This test is designed to elucidate this mechanism and to determine whethe other chemical agents are capable of acting similarly. Normal rats maintained on a

diet containing propylthiouracil are the test animals. The propylthiouracil isolates the iodide-concentrating step of thyroidal hormone biosynthesis. When radioactive iodide is administered, the only form found in the thyroid gland of such rats is inorganic iodide, and simultaneous measurement of the blood and thyroid iodide permits the calculation of the concentrating capacity of the gland. Utilizing this test, various chemicals have been assayed for their ability to alter the concentrating capacity of the thyroid gland for iodide. Those chosen have been used because of their oxidative or reductive nature. To the present time 2 agents have been found which reproduce the action of thiocyanate ion - sodium perchlorate and sodium chlorate, both oxidizing agents. Repeated tests have shown that their effect is reproducible and time-versus-effect curves have established their duration of action. The data obtained suggest that these agents act by virtue of their capacity as oxidizing agents and that the concentrating capacity for iodide of the thyroid gland is dependent upon the maintenance of an intact redox potential within the gland.

Quantitative Aspects and Locus of Peripheral Action of Thyroid Hormone. A completely myxedematous animal has no circulating thyroid hormone. It has also been shown that the oxygen utilization of either intact myxedematous animals or their isolated tissues are reduced from normal. If radioactive thyroxine of known specific activity be injected into such animals, and the amount of hormone taken up by a single tissue be determined , the absolute amount of hormone uptake by the tissue can be determined. The injection of thyroid hormone results in an increased oxygen uptake by the isolated tissues of myxedematous animals. If both the hormone accumulation by an isolated tissue and its influence on oxidative metabolism of the tissue be simultaneously determined, a quantitative relationship can be drawn between the two.

Work is being carried out in rats, using the paired hemidiaphragms for the 2 determinations. The oxygen uptake of the diaphragm of both normal and myxedematous rats has been defined. Actual simultaneous measurements of hormone uptake and its influence on oxygen uptake of myxedematous rat diaphragms will be made.

A latent period is seen before the action of thyroid hormone on oxygen uptake occurs, although the hormone combines quickly with peripheral tissues. The course of this latent period will be investigated by means of differential centrifugation of homogenized diaphragm in which hormone uptake has occurred. An attempt will be made to correlate the time of onset of hormonal action with the intracellular locus of the hormone.

Anion Concentration Mechanisms The initial stage in thyroid synthesis is the accumulation or trapping of iodide against a concentration gradient. Thus inorganic iodide is concentrated within the thyroid gland prior to oxidation of iodide to iodine and thyroid hormone formation. It has been hard to dissociate the concentrating and organic binding process within the gland except by pharmacologic means. It was anti-

agents can suppress the S/P ratio to below 10 but never to unity. Significantly these are the agents which also wash out iodide from the thyroid iodide trap. Administration of TSH depresses the salivary-concentrating capacity for iodine. Whether this is a primary effect upon the salivary transport mechanism for iodide or secondary to alterations in blood halide interrelationships remains to be elucidated.

Acute disruption of the salivary anion transport metabolism will be attempted in humans by pharmacologic means. A single experiment in the rat revealed that the ability of the stomach to concentrate iodine could be depressed by administering TSH. However this method is technically more difficult than the salivary approach.

Quantitative Studies of Thyroidal Dynamics. The biosynthesis of thyroid hormone occurs in 2 stages: 1st the concentration by the thyroid gland of inorganic iodide from the serum, and 2nd the oxidation of iodide to iodine and its binding to protein.

A series of equations have been developed which make it possible by means of the administration of radioactive iodine intravenously, followed by the determination of accumulation gradient over the next 3 hours and the analysis of blood iodide activit to determine the absolute level of the concentration gradient of the human thyroid glan for iodide, and the absolute rate of conversion of thyroidal iodide to organically-boun hormone. The latter function has never before been evaluated quantitatively in animals or man. The relationship between the 2 has not before been evaluated, as the only previous method of determining the concentration gradient was by the administration of propylthiouracil, which blocks hormone formation. This itself may alter the concentrating capacity of the thyroid for iodide.

Thirty patients have been studied, utilizing this technique. Normal values are being defined. Both hypothyroid and hyperthyroid patients have been studied, and also patients receiving TSH, propylthiouracil, and iodide. Preliminary data indicated a good correlation of the derived constants with clinical state. Utilizing these equations it is also possible to calculate thyroidal plasma-iodide clearances, thyroidal body-iodide turnover times and iodide distribution space. Values for the latter functions agree well with those obtained by other investigators utilizing other methods.

A greater number of normal and diseased patients will be studied. When normal and abnormal values have been defined, the method will be used to determine the locus of action of various hormonal and medicinal agents which affect the thyroid. In additi for the 1st time quantitative assessment of the potency of antithyroid agents can be ma

Peripheral Utilization of Thyroid Hormone. This experiment utilized an in-vitro system to study the rate of uptake of thyroid hormone by the isolated rat diaphragm. I initial experiments to ascertain whether a definite uptake of hormone occurs when diaphragmatic tissue is incubated with radioactive thyroxine of known specific activity, definite binding of hormone was observed. Bound hormone could not be removed by repeated washings. Control experiments with radioactive inorganic iodide revealed no such irreversible union.

Initial studies done in normal rats and in rats made myxedematous with propylthiouracil showed that the absolute amount of hormone uptake in both groups was directly proportional to the thyroxine concentration in the incubating medium, and was equal in magnitude in both groups.

This preparation will be utilized as a means of studying the effect of various hormonal and environmental influences on the uptake of thyroid hormone by peripheral tissue. The rate of uptake may represent the rate of peripheral utilization of this hormone.

The Extrathyroidal Synthesis of Thyroid Hormone. Studies in 12 euthyroid individuals have demonstrated that butanol-extractable I^{131} fraction can be elevated by the administration of exogenous TSH. Attempts have been made to dissociate this rise from the thyroidal effects of TSH by studying the effects of TSH in 6 patients with postoperative hypothyroidism or hypothryoidism secondary to I^{131} ablation of the thyroid gland. In each of these TSH elevated the I^{131} butanol-extractible fraction without altering the uptake of radioactive iodine over the neck. Absence of demonstrable augmentation of thyroid function in these subjects would suggest an extrathyroidal focus of TSH action. These results are in marked contrast to those observed in 2patients with primary myxedema. Here administration of TSH did not augment the plasma level of butanol-extractable I^{131}.

Twelve rats have been thyroidectomized by graded doses of I^{131}. The establishment of complete myxedema is awaited.

The Effect of Hormonal and Pharmacologic Agents upon the Ability of the Thyroid Gland of Rats to Concentrate Inorganic Iodide. This study was undertaken in order to determine the factors which control the capacity of the thyroid gland to concentrate inorganic iodide. This function was isolated from the subsequent stage of hormone function by the administration of propylthiouracil.

Intact animals receiving propylthiouracil were studied. The concentrating capacity for inorganic iodide of the thyroid gland was found to be 200-250 times the concentration in the serum. Subsequent groups of animals were given varying doses of thyroxine in order to suppress endogenous thyrotropic (TSH) secretion. In these animals the concentrating capacity for iodine (concentration gradient) was markedly reduced from that of the previous group. Administration of exogenous TSH restored the concentration gradient of thyroxine-treated animals toward that of animals not receiving thyroxine.

Similar studies were then done in about 250 hypophysectomized mice, also given propylthiouracil. The concentration gradient for iodide was only 1/20 that of intact animals similarly treated. The administration of exogenous TSH increased the concentration gradient to the level found in intact animals. A graded response of the concentration gradient was obtained by graded doses of TSH. The response to TS H was extremely sensitive, minimal responses being obtained with .04 mg. of hormone. The concomitant administration of thyroxine reduced the response of hypophysectomized animals to exogenous TSH, and also reduced even further the already low level of the concentration gradient in hypophysectomized animals not receiving TSH.

Next the influence of increasing serum inorganic iodide concentrations on the concentration gradient was studied. Large doses of iodide reduced the concentration gradient of hypophysectomized animals, either untreated or treated with TSH. Larger amounts of iodide were required to suppress the gradient in animals given TSH than in those not so treated.

An experiment is under way in which the influence of cortisone on the concentration gradient of hypophysectomized, propylthiouracil-treated animals will be determined. It can thus be ascertained whether the direct inhibitory effect of cortisone on the thyroid gland occurs via an action upon the concentration of iodide or on its conversion to hormonal iodine.

II. P^{32}

Clinical Use of P^{32} . Three patients with metastatic carcinoma of the breast have been treated with divided oral doses of P^{32}. Results in 2 were moderate to good relief from pain. One of these cases who had large cutaneous lesions at the site of surgery showed a marked decrease in the size of these lesions. The 3rd responded poorly and therapy was stopped after 7.8 mc. orally to switch to irradiation. One patient is under therapy.

P^{32} RBC volumes continue to be run on a routine basis, using the liquid counting tubes.

A series of polycythemia vera cases have been controlled successfully with P^{32} Susceptible, widespread lymphosarcoma cases have been treated with P^{32} with fair temporary results. P^{32} proved useful in determining RBC volume using a modification of the Reeve-Veall technique.

Effects of Antibiotics on Radiation Syndrome

BRIEF. The purpose is to study the effects of ionization on animals and human subjects, using antibiotics in therapy.

BACKGROUND. Sixteen dogs were irradiated with 300 r at 250 KV, 30 MA, using 1 mm of aluminum plus 0.5 mm copper added filtration. They were divided into 9 controls receiving no medication and 7 receiving aureomycin. A 78% mortality was seen in the controls at the end of 37 days and a 71% mortality in the treated animals. It has been noted that dogs receiving medication live on the average of 2-4 days longer than the control group.

PROGRESS. A series of dogs were exposed to an LD_{80} dose of irradiation, using 250 KV 30 MA with added filtration of 1 mm. aluminum and 0.5 mm. copper. The dogs were exposed a half of the dose to each side. One-half had been dehydrated for 3 days immediately prior to radiation. The results following a 30-day observation period showed no difference in the mortality rates of the 2 groups.

Tissue Response to Radiation

BRIEF. These experiments are designed to study the effects of ionization on cell metabolism.

BACKGROUND AND PROGRESS. Normal water, fat and nitrogen content of normal marrow has been determined. Alterations of lean marrow mass as a function of age, sex, and marrow site have been studied in 10 rabbits. Duplicate Kjeldahl nitrogens on all preparations have shown an overall check of 1.31%. Eleven experiments have been completed on the in-vitro respiration of normal rabbit marrow and Q_{O_2} (N) values have shown fair uniformity in this regard. Rate curves have been for the most part linear.

In view of some variability of marrow respiration from animal to animal and of a rather unique opportunity to work with 2 strains of *E. coli* having somewhat different biochemical characteristics comparison of effects of UV and x-ray irradiation on these bacteria has been undertaken in an attempt to localize biochemical sites of action of irradiation.

Ten experiments of the dose-survival curve type indicated that the 2 strains have markedly different susceptibilities to UV irradiation. Several experiments on x-ray sensitivity yielded conflicting results and require further investigation. This attack at present would seem productive of results subject to fewer variables and is technically more feasible than the intact animal irradiation studies. The latter will be continued when the bacterial experiments have pointed up the more likely sites of biochemical interference.

Radiation Mortality in Mice in the Neonatal Period

BRIEF. Mice are being irradiated with the object of discovering factors affecting radiation mortality in the neonatal period; influence of weaning, age, weight, sex, and endocrine patterns.

BACKGROUND AND PROGRESS. Factors Affecting Radiation Mortality in Mice in the Neonatal Period - Influence of Weaning, Age, Weight, Sex, and Endocrine Patterns. Studies were done in approximately 1200 mice relative to the influence of such factors intrinsic to the irradiated, but otherwise untreated, animal on radiation mortality; It has been found that irradiation deaths are negligible in mice irradiated before approximately 21 days of age, increase to a peak in animals irradiated at 30 days, the approximate time of puberty and thereafter gradually decrease. Weaning of mice at 21 days rather than the usual 30 days deleteriously influences their subsequent course, as indicated by both mortality and growth rates. Males are more sensitive than females to the toxic effects of x-irradiation, as indicated by both postirradiation growth and mortality rates, the ratio of deaths in males to females being 2.3/1 in animals irradiated postpubertally. No such sex difference was noted in animals irradiated prior to puberty. As compared to sham operation, prepubertal orchiectomy was markedly protective to mice irradiated during the pubertal or postpubertal period. The mortality rate following irradiation of prepubertally castrated mice was restored to that

and are now under observation.

REFERENCE.

"Intrinsic Factors Affecting Radiation Mortality in the Mouse." Ingbar, S. H., and Freinkel, N., Fed. Proc., March 1952.

RESEARCH AND DEVELOPMENT PROJECT CARD (NEW PROJECTS)	2. SEC. U	3. PROJ. NO. 6-60-01-001
1. PROJECT TITLE: Stress		4. 5. REPORT DATE 30 June 52
6. BASIC FIELD OR SUBJECT: Internal Medicine	7. SUB FIELD OR SUBJECT SUB GROUP: Investigation, Authorized	PO-15
8. COGNIZANT AGENCY: Med Res & Dev Bd	12. CONTRACTOR AND/OR LABORATORY: See below	CONTRACT/W. O. NO.
9. DIRECTING AGENCY: The Surgeon General, DA		
10. REQUESTING AGENCY	13. RELATED PROJECTS	17. EST. COMPL. DATES: RES. / DEV. / TEST / OP EVAL
11. PARTICIPATION AND/OR COORDINATION	14. DATE APPROVED: 9 July 1951	
	15. PRIORITY: 1-C 16.	18. FISCAL EST'S. P_Y: 52 614M; 53 667M; 54 760M
19.		

20. REQUIREMENT AND/OR JUSTIFICATION

Recent advances have indicated that stress, in the form of mental trauma, extremes of temperature, body injury, etc., produces definite changes in body mechanisms apart from the usually recognized effects of these agents. It is imperative that methods be developed to measure the non-specific effects of stress on the soldier, and further, the ways and means of altering this effect in the direction of better body economy.

21. BRIEF OF PROJECT AND OBJECTIVE

a. Brief: The objective is to determine the basic mechanisms, which are operative in the production of the non-specific effects of stress; to devise tests to measure this effect; and to develop practical methods for altering these effects in the direction of improved body efficiency.

Soldiers are constantly subjected to a wide variety of stresses both mental and physical, which commence with the abrupt change from civilian to military life. These stresses are ever recurring throughout the periods of training, and reach their greatest magnitude on the field of battle. These unusual stressful situations, in many ways peculiar to the soldier, include such things as anxiety, fear, exposure to extreme variations in environmental conditions, fatigue, diseases, malnutrition, and injuries. Recent studies have shown that these factors, in addition to the known and well recognized effects peculiar to the specific agent, also produce a profound but non-specific effect on the total organism. There is some evidence to indicate that the specific and non-specific effects of these factors on the total organism, may be

Project No. 6-60-01-01
Contract No. MD-255

NOT FOR PUBLICATION
Report Date 30 June 1952

Stress

Dr. Oscar Hechter
Worcester Foundation for
Experimental Biology
Shrewsbury, Mass.

BRIEF. The objective is to study mechanisms of steroidogenesis and the influence of stress upon corticosteroidogenesis.

BACKGROUND AND PROGRESS. The perfusion apparatus developed in this laboratory has been redesigned; methods for the extraction of steroids from perfusion media have been compared; and the influence of incubating steroids in oxygenated blood at 38° C has been investigated. The latter point is of special importance since perfusion work here, for the most part, used blood as perfusion medium. Solvent extraction procedures (ether-chloroform 4:1 or ethyl acetate) are preferable to the charcoal method employed previously for certain problems, while for others involving small volumes of blood the dialysis procedure was most satisfactory. Studies involving corticosteroid incubation in oxygenated blood at 38° C revealed that the bulk of steroid added is recovered unchanged, although the presence of new products formed in trace amount is indicated.

Upon completion of the control studies, perfusion experiments were undertaken with radioactive cholesterol, cholestenone, and progesterone (all ring-labeled with C^{14}) in the presence and absence of added ACTH. The results demonstrate conclusively that cholesterol but not cholestenone is transformed to adrenal corticosteroids and that progesterone is converted to a variety of steroid ketols in addition to the expected products, 17-hydroxycorticosterone and corticosterone. The effect of ACTH upon the foregoing reactions has been evaluated by measuring the total incorporation of radioactivity into corticosteroid from the C^{14} substrates employed.

FUTURE. Further work with cholesterol and progesterone is necessary, but the indications strongly suggest that ACTH acts in corticosteroidogenesis at a point prior to progesterone.

Dehydroepiandrosterone will be perfused through adrenals in the presence and absence of ACTH.

Further work on progesterone will be undertaken so that the pathway leading to compounds F and B may be more clearly elucidated.

Project No. 6-60-11-17
Contract No. MD-109

NOT FOR PUBLICATION
Report Date 30 June 1952

Studies in Sulfur Metabolism

Dr. Laurance Kinsell
Highland Alameda County Hospital
Oakland, California

BRIEF. The objective is to determine the rate of wound healing in humans under normal and abnormal conditions, using isotopic sulfur as a tracer; to evaluate the metabolism of methionine, cystine, glutathione, and other organic sulfur-containing materials under specific experimental conditions in human subjects; and to determine the rate of incorporation of methionine and cystine into specific tissues and organs.

BACKGROUND. The use of S^{35}-labeled methionine as a tool for the study of protein metabolism having been established through previous research, early work on this project centered around 2 subtasks: evaluation of the metabolic defect in 2 patients with idiopathic hypoproteinemia by the use of S^{35}-labeled methionine, and evaluation of the effect of ACTH upon protein metabolism with the use of the same substance.

The details given in June 1951 were summarized as follows: It appears that hypercatabolism of plasma albumin is the fundamental etiologic defect in this symdrome and that the reason for this hypercatabolism is unknown. Anabolism of plasma albumin is in no way impaired and is probably significantly accelerated in an effort to compensate for the hypercatabolism. The use of high protein intake plus testosterone propionate appears to improve the clinical status of the patient but fails completely to bring the albumin level to normal.

Studies indicate that in the "nephrotic": The rates of both albumin and globulin synthesis are greatly increased over the normal; the rate of synthesis of albumin and the rate of urinary loss of albumin are almost identical (this is true with or without albumin infusion); the S^{35} content of the urinary protein is identical with that of the serum albumin; and ACTH caused a rapid cessation of albuminuria and a return to normal of plasma protein levels.

What was believed to be a satisfactory technique for evaluation of rate of wound healing with the aid of S^{35}-labeled methionine was described in March 1952.

PROGRESS. Further studies using S^{35} for the evaluation of the rate of wound healing were done. The actual technique is now very well standardized and gives highly reproducible results in different groups of animals as well as within any given group. To date, studies designed to evaluate the effect of cortisone and ACTH upon the rate of wound healing have failed to show any significant effect. Studies now under way include prolonged pretreatment of animals with ACTH and cortisone. It is believed that the lack of effect is referable to inadequate absorption of cortisone and to inadequate duration of ACTH-administration in well-nourished animals.

FUTURE. These studies of humans and animals will be continued and will include evaluation of several hormonal agents known to affect protein metabolism.

RESEARCH AND DEVELOPMENT PROJECT CARD (NEW PROJECTS) | 2. SEC. U | 3. PROJ. NO. 6-60-13-012

1. PROJECT TITLE
Bacterial & Fungous Infections of the Skin

4. REPORT DATE 30 June 52

6. BASIC FIELD OR SUBJECT
Internal Medicine

7. SUB FIELD OR SUBJECT SUB GROUP
Therapeutics

8. COGNIZANT AGENCY
The Surgeon General, DA

9. DIRECTING AGENCY
Med Res & Dev Bd

10. REQUESTING AGENCY

11. PARTICIPATION AND/OR COORDINATION

12. CONTRACTOR AND/OR LABORATORY
See below

CONTRACT/W. O. NO.

13. RELATED PROJECTS

14. DATE APPROVED
15 January 1949

15. PRIORITY 2A | 16.

17. EST. COMPL. DATES
RES. Cont.
DEV.
TEST
OP EVAL

18. FISCAL EST'S.

FY	
52	46 M
53	105 M
54	110 M

19.

20. REQUIREMENT AND/OR JUSTIFICATION

Chronic eczematoid dermatitis comprises by far the largest source of dermatologic disability among Army personnel. It is necessary to study the factors leading to superficial bacterial and fungous infections of the skin, and subsequent chronic eczematization, with particular reference to environmental conditions obtaining among military personnel; and to develop methods of preventing and alleviating such infection and chronic sensitization.

21. BRIEF OF PROJECT AND OBJECTIVE

a. Brief: The objectives are to study: factors leading to chronic disabling dermatitis in military personnel, new fungicides superior to those now used by the Army, and methods of screening such new agents.

b. Approach: A broad attack upon the problem of infection and chronic sensitization of the skin includes: (1) thoroughgoing laboratory studies in conjunction with (2) clinical studies on adequate patient material, including military personnel as indicated. New fungicides must be screened in order to find preparations more effective than those now in use.

Paragraph 12, Contractor and/or Laboratory

(a) The Johns Hopkins University - Dr. Maurice Sullivan

To study new fungicidal preparations in an attempt to find compounds that are more effective than the fungicides now in use by the Army Medical Service

d. Other Information: None.

e. Background and/or Progress: Dr. Pillsbury considers the chief etiologic factors in disabling chronic dermatitis to be bacterial and fungous infections, sensitivity to these organisms, epidermal and dermal sensitivity induced by treatment, and disorders of sweating preceding or following the initial evidence of dermatitis. (Not all the conclusions reported below resulted entirely from specific studies under this grant.) (1) Apparently there is a slow, steady increase in the percentage of the population sensitive to penicillin, caused by its use in various infections over a period of years. Dermatologic disability in the Army could be reduced if penicillin were not used in mild intermittent, probably self-limited, infections; and if patients about to receive penicillin were queried as to previous reactions to it. (2) Rhus extract, whose value in acute poison ivy dermatitis has never been proved, should now be given for the treatment of this condition. Prophylactically it is of uncertain value; if employed, it should be given under the supervision of a competent dermatologist or allergist. (3) Considerable disability arises from the use of preparations to control itching, none of which is really satisfactory. Compounds related to novocaine are the chief sensitizers. A local anesthetic not related to novocaine (Quotane) gave satisfactory relief from itching in about 85% of 700 patients, with no frank epidermal sensitivity. (4) The treatment of "fungous" infections continued to be unsatisfactory in Army practice because of frequently-erroneous diagnoses and ineffective fungicides. Newly-available compounds are tested clinically. (5) The specific stain for fungi based on the Hotchkiss-McManus method was modified to be usable in any laboratory. The diagnosis of fungous infection should be proved microscopically before being accepted in Army hospitals in order to avoid almost certain disability from erroneous therapy. The "cure" of fungous infections is obtained principally by the removal of infected material, by local immune responses or mechanico-chemical methods. (6) Various antibacterial agents for local application are studied; none is entirely satisfactory. Neomycin is promising but very expensive. (7) X-ray therapy, which is commonly used in treating superficial inflammatory eruptions, has been grossly overused, with serious sequelae. The only known controlled study was conducted here to determine its exact value. The beneficial effects of superficial x-ray therapy are evanescent if the primary cause of the eruption still exists. It is concluded that x-ray therapy for such eruptions is rarely justified overseas and then under only the most competent supervision, and in U. S. Army hospitals should be restricted further. Continuing studies with low-voltage (Grenz) therapy suggest that it is as effective as conventional x-ray therapy in these eruptions and that the margin of safety is much greater. Patients who have received Grenz-ray therapy are being followed for sequelae. (8) ACTH and cortisone, while often immediately beneficial in their effects on eczematous eruptions, almost always are followed by severe recurrences, frequently more marked than the original eruption. In allergic skin reactions which probably will be self-limited, such as severe penicillin reactions, both

preparations rapidly cure in most cases and their use is justified. This is not true in certain severe general medical conditions in which the skin, is often affected, such as acute disseminate lupus erythematosus, polyarteritis, nodosa, etc. Cortisone ointment (25mgm per gm in a carbowax base) was concluded to be valueless in a controlled experiment in 25 patients.

Studies on the Causative Organism of Trichomycosis Axillaris. In 100 consecutive clinical cases examined, 28 cases of trichomycosis axillaris were found. Corynebacteria were isolated from all 28 cases, and from 3 normal cases. The cultural, microscopic, and growth characteristics, mouse pathogenicity, and antibiotic sensitivities of these organisms were reported. The name Corynebacterium tenuis was proposed for the organism causing trichomycosis axillaris in Philadelphia and vicinity.

Are Fungous Infections increasing as a Result of Antibiotic Therapy? Candida albicans regularly emerges in abundance in the mouths and gastrointestinal tracts of those receiving wide-spectrum antibiotics. The isolation of this organism in the presence of some untoward side-reaction such as glossitis or stomatitis is not tantamount to a diagnosis of moniliasis. Evidence was presented that the stigmatization of the mucous membrance reactions observed in association with antibiotic therapy as moniliasis probably is unfounded. The wide-spectrum antibiotics did not enhance the growth of C. albicans in vitro nor potentiate mycotic disease in animals with experimental moniliasis, blastomycosis, histoplasmosis, coccidioidomycosis, and sporotrichosis.

Studies of the apocrine sweat gland offer the greatest hope for a sound understanding of a critical factor in a problem of prime importance to the armed services, otitis externa, and in eruptions of the axilla and genitalia. Progress was being made in a study of the etiologic role of the sweat gland in several common disabling and chronic skin diseases. Physiology of the Human Axillary Apocrine Sweat Gland. Apocrine sweating could be stimulated by emotional stimuli of significant intensity. Apocrine sweating was produced by epinephrine and nor-epinephrine. Apocrine sweating was not produced as a result of acetyl choline, pilocarpine, or thermal stimulation. The apocrine secretion was seen to appear against an external pressure of 225 mm. of mercury. Such sweating could be produced in adults but not in children; there was a reduction in quantity in the older age group. The apocrine sweat gland unit showed a definite refractory period, both to neural as well as chemical stimulation. It has been postulated that apocrine sweating is under the control of adrenergic fibers of the autonomic nervous system. Localized Chromidrosis, A

RESEARCH AND DEVELOPMENT PROJECT CARD (NEW PROJECTS)	2. SEC. U	3. PROJ. NO. 6-63-01-006
1. PROJECT TITLE: Oral Disease		4. REPORT DATE: 30 June 52
6. BASIC FIELD OR SUBJECT: Dentistry	7. SUB FIELD OR SUBJECT SUB GROUP: Investigation Authorized - PO-14	
8. COGNIZANT AGENCY: The Surgeon General, DA	12. CONTRACTOR AND/OR LABORATORY: See Below	CONTRACT/W. O. NO.
9. DIRECTING AGENCY: Med. Res. & Dev. Bd.		
10. REQUESTING AGENCY: Medical Service, DA	13. RELATED PROJECTS: None	17. EST. COMPL. DATES: RES. continued; DEV.; TEST; OP EVAL
11. PARTICIPATION AND/OR COORDINATION: None	14. DATE APPROVED: 9 July 1951	FY 18. FISCAL EST'S.
	15. PRIORITY: 2B 16.	52 336M
19.		53 303M
		54 400M

20. REQUIREMENT AND/OR JUSTIFICATION

To reduce oral disease which affects 98% of the military age group, resulting in time-loss for treatment. This study requires investigation in all fields of the science of dentistry. Studies in the basic causes as well as methods of treatment are essential if any substantial reduction in dental casualties is to be effected.

21. BRIEF OF PROJECT AND OBJECTIVE

a. Brief:

The overall objective is to investigate the causes, treatment, and epidemiology of dental caries, periodontitis, and other oral diseases which affect military personnel. By following this objective it is contemplated that such an effort should, as investigations progress, result in improved methods of treatment of dental and oral lesions. Further, as information on etiology and effects of treatment become available, the number of dental lesions can be reduced, and the cost of dental care proportionately. The Steelman report to the President stated, "The small group of projects on dental and oral disease by the federal agencies reflects the general neglect of dental research, and the need of training investigators in the field." Special effort has since been made by the military and dental educational institutions to train dental research personnel.

The Rockefeller Foundation stated, "Although America leads the world in dentistry, it is a leadership based more upon ingenuity of a mechanical sort

coordinated by the Dental Research Advisory Committee into a balanced program. Studies to include:

(1) Diseases of the tooth.

(a) The etiology of dental caries including the bacteriology and biochemistry of early and terminal lesions.
(b) Treatment of the carious lesion with emphasis on cavity preparation for the insertion of filling materials.
(c) Physical properties of tooth structure.
(d) Analyses of materials used as dental restorations to include their physical properties, effect on tooth structure, and their ability to prevent further destruction of the tooth.
(e) The evaluation of present data, and further investigation into the use of fluorides and other substances, known and being developed, in the treatment of dental caries.

(2) Diseases of the periodontium.

(a) Histochemical, anatomical, histopathological, bacteriological, and nutritional studies into the etiology of periodontal disease.
(b) Methods of treatment and means for prevention of periodontal disease.
(c) Bacteriological and dietary studies into the nature of calculus formation. This is one of the primary causative factors of periodontal disease.

(3) Diseases and injuries of the oral mucosa and jaws.

(a) The classification and definition of oral keratotic lesions.
(b) The oral manifestations of nutritional deficiencies.
(c) Studies in facial growth and development.
(d) Methods of early diagnosis in degenerative diseases.
(e) Development of methods for early diagnosis and more effective methods in the treatment of acute and chronic inflammatory lesions.

(4) Dental Materials, instrumentation, and clinical facilities.

(a) Research in the properties of dental materials.
(b) Improvement of specifications of existing dental materials.
(c) Studies to improve present restorative procedures.
(d) Methods to increase the efficiency of instrumentation, clinical facilities, and patient handling.

c. Subtasks

(1) Army Medical Service Graduate School is investigating the bacteriological and biochemical aspects of dental caries.

(2) The Armed Forces Institute of Pathology is investigating the etiology and methods of classification of periodontal disease.

(3) The University of California under Dr. Herman Becks is studying the effect of the topical application of fluorides and other substances on the control of dental caries in young adults.

(4) The University of Indiana under Dr. Harry G. Day is studying the effect of fluoride substances on the nutritional requirements of the Lactobacillus acidophilus.

(5) The University of Illinois under Dr. Milton Engel is undertaking a histochemical analysis of the gingival tissues in relation to periodontal disease.

(6) The Beth Israel Hospital under Dr. Henry Goldman is studying the mechanics of pocket formation in periodontal disease by histopathological methods.

(7) The University of Illinois under Dr. Joseph Weinmann is conducting cytologic studies on oral epithelium in regeneration, and in benign and malignant neoplasia.

(8) Walter Reed Army Hospital Dental Service is investigating the hard tissues of the masticatory mechanism by cephalometric means, to accomplish a more adequate restoration to normal function.

(9) The University of Rochester under Dr. Basil G. Bibby is studying the role of bacteria and their products in non-specific gingival disturbances.

(10) The University of Illinois under Dr. William W. Wainwright is conducting an autographic determination of the ability of various bacteria and other substances involved in the production of the carious lesion to penetrate dental structures and filling materials, by the use of radioactive tracers.

(12) Georgetown University School of Dentistry under Dr. Gustav Kruger is evaluating superficial keratotic lesions of the oral regions by clinical and histologic methods.

(13) The University of Michigan School of Dentistry under Dr. Donald Kerr is studying the effect of inhalation of air borne particles resulting from the use of abrasive technique in cavity preparation.

(14) The University of Illinois under Dr. Isaac Schour is investigating the response of the dental pulp to the traumatic and chemical irritants and to the various types of cavity preparation and filling materials.

(15) The University of Michigan School of Dentistry under Dr. William Mann is investigating the possibility of utilizing fluid turbine power in the dental handpiece.

(16) At the National Bureau of Standards, research is being conducted on the physical properties of dental materials, restorative procedures, and of increasing efficiency in restoration techniques.

(17) The University of Southern California under Dr. Hollenback is attempting to determine more effective means of utilizing amalgam alloys by correlating laboratory data with clinical procedures.

(18) The Eastman Dental Dispensary under Dr. Finn Brudevold is studying Dental Adhesives.

(19) Indiana University under Dr. Robert B. Fischer is studying the physical structure of dental enamel and bones, with particular emphasis on surface structure.

(20) The University of Nebraska under Dr. Ralph L. Ireland is conducting an investigation to determine the stress and strain patterns which may be set up in the tooth as a result of various types of class II cavity preparations for silver amalgam restoration.

(21) Madigan Army Hospital is conducting a study of Periodontal Disease in Persons between the ages of 20 and 23.

(22) Tufts College Dental School under Dr. C. D. Marshall-Day is conducting an epidemiological study of periodontal disease.

(23) The University of Rochester under Dr. Elliott A. Maynard is conducting experiments in the development of dental caries in the Syrian Hamster.

(24) The University of Indiana under Drs. Joseph C. Muhler and William G. Shafer is studying the relationship between the endocrine system and dental caries.

(25) Percy Jones Army Hospital is studying electronics as a diagnostic aid in dentistry.

(26) Northwestern University under Dr. Eugene W. Skinner is studying new designs and greater efficiency of dental tools at various speeds.

(27) New York University under Dr. Otto R. Trautz is studying the preparation and chemical analyses related to the apatites of dental enamel.

(28) The University of Indiana under Dr. Grant Van Huysen is studying the response of dentin and pulp to the cutting of cavities in the tooth by airbrasive compared to the response to cavity preparation with the present standard procedures.

(29) The University of Pittsburgh under Dr. S. Wah Leung is studying the influence of salivary secretion on calculus formation as studied by the use of radio-isotopes.

(30) The following additional subtasks are planned for FY 1954

(a) Treatment of dental caries in young adults by the use of chemical agents such as fluorides, colloidal silver, colloidal iodine, etc.

(b) Treatment of periodontal disease by more effective utilization of corrective procedures.

(c) Studies to improve treatment methods related to dental caries.

(d) Improvement of oral surgical techniques, instruments, and materials in the treatment of oral disease, and traumatic injuries.

(e) Studies into more effective methods of oral diagnosis.

(f) Therapeutic x-radiation in dentistry.

(g) Histological studies of tooth structures using radioactive isotopes.

d. Other Information:

The following research proposals are now under consideration by the Dental Research Advisory Committee of the Army and will be activated as soon as approved.

(1) The University of Alabama under Dr. W. Ward Pigman is to study the nature and amount of carbohydrates in human saliva and to study their relationship to the etiology of dental caries.

(2) The University of Minnesota under Dr. Wallace D. Armstrong is to develop improved methods for determination of microquantities of fluoride in biological materials (foods, urine, blood, soft tissues, bone and teeth) and to apply these methods to the study of the "normal" metabolism of this element.

(3) The Walter G. Zoller Memorial Dental Clinic of the University of Chicago under Drs. Thomas B. Coolidge and Frank C. Besic is to study the analysis of factors associated with the in-vitro production of caries of the enamel.

(4) Indiana University under Professor Ralph Phillips is to study the electrical and thermal conductivity of dental cements and restorative materials.

(5) The University of Illinois under Dr. Robert F. Nystrom is to synthesize a series of compounds of dental interest that will be helpful in research on dental pathology and dental materials. Radioactive acrylic monomer is the first compound to be synthesized.

e. Background and/or Progress:

Avery has studied the formation of teeth in swine from the time of initiation of eruption and in man from the time of initiation to amelogenesis. The Bell stage of development furthermore is characterized by a contact of the inner and outer enamel epithelium which occurs just before dentinogenesis begins. Later during amelogenesis the top of the enamel organ is evaginated exposing the amelogenic area to the vascular tooth crypt area. In later stages a vascular network is established in close contact with the ameloblasts and the cells of the stratum intermedium rotate 90° during this period so that 1 end of these cells is in contact with the capillaries and the other with the neighboring ameloblasts. During eruption the stratum intermedium cells appear to revert to their original plane perpendicular to the ameloblast and to proliferate, forming cellular bridges between the reduced enamel organ epithelium and the gingival epithelium.

In man, Avery found that the labial and the dental lamina develop at approximately the same time. The bell stage of tooth development does not occur in man or pig until the stratum intermedium (overlaying the inner enamel epithelium) contacts the outer enamel epithelium. Histo-differentiation of the formative cells begins slightly before this time in both species but these cells do not mature until complete

invagination occurs. Dentinogenesis occurs at the apex of the completely invaginated inner enamel epithelium in both man and pig. At the beginning of amelogenesis in man the upper convexity of the enamel organ is evaginated so that 1/3 to 1/2 of the amelogenic area is in contact with the tooth crypt and the remainder is in contact with the stellate reticulum. Evidence is presented by Avery that the 3 lobes of the maxillary central incisor are indicated at an early stage of tooth formation in man.

Becks has been studying the effects of fluorides on caries in the young adult. Up to July 1950, 548 students had received 4 fluorine applications preceded by thorough dental examination, dietary history, 2 Bacillus acidophilus counts, roentgenograms, and prophylactic treatments. Of these, 68 students of the 1st group, started in April 1949, were re-examined for caries activity after 1 year. Of this group, in May 1951, only 58% responded to the request for re-examination, a large number having joined the armed forces during the year. During the 1st 4 months of 1952, 6 groups of students who enrolled 2 and 3 years ago in the fluorine administration program were recalled, re-examined, and re-roentgenographed. One hundred and one students had their 1st-year dental check and belong to 2 groups which were started in 1951. One hundred eighty-nine had the 2nd-year re-examination; they were started in 1949 and 1950.

With each re-examination a saliva specimen is obtained for the determination of the Lactobacillus acidophilus index as well as roentgenographic bite-wing pictures. All dental examinations are made by 4 staff members who have been with the project since initiation. An estimated 400 students would receive their 2nd-year check and approximately 500 their 3rd-year check during 1953, leaving an estimated 500 students for 1954.

Day has devoted considerable effort during the past few months to studies aimed at the further reduction of the level of fluorine in the components of the experimental diet. Some success has been achieved, but the methods are time-consuming. Because a means of producing dietary components extremely low in fluorine is so important, this work is being continued.

The ability of beryllium to form a soluble complex with fluorine, under certain conditions, led to an attempt by Day to apply this property to the purification of dietary components. All such efforts have been unsuccessful. None of the various methods of dialysis tried has shown promise. The best methods for starch appear to involve repeated extraction with dilute acid. The best preparations of casein have resulted from repeated precipitation from slightly alkaline solutions and washing with large amounts of water. Unfortunately, the best results are obtained when the batches do not exceed

connective tissues which have been studied to this time by Engel behave as components of a continuum which vary from loose to densely aggregated, negatively charged colloid. The inter-action of positively charged ions with the colloid is now virtually predictable upon the determination of the density of colloid from electrochemical data. Each tissue also has a lability which results in a variable range from "looseness" to "tightness" under hormonal and other influences. The distribution of colloid, water, and electrolyte between a colloid-rich, water-poor phase and a water-rich, colloid-poor phase has been postulated.

Engel believes that the serum mucoprotein level and the urinary excretion of mucoprotein largely represent residues from the turnover of connective tissue ground substance. Additional studies must be done to test the hypothesis. Engel finds that the generalized alveolar bone loss in periodontosis appears to be unrelated to local causes. Experimental studies with parathyroid extract lead Engel to consider that active extensive bone resorption due to any cause may be reflected by an elevation of the serum mucoprotein level due to solution of residues of the mucoprotein bone matrix.

In such conditions as dentinogenesis imperfecta, odontones, pulp stones, internal dentin resorption, etc., the dentin is characterized by an altered chemical reactivity which leads to a more intense reaction with the periodic acid-leuchofuchsin reagent according to Engel. These histochemical findings are interpreted to indicate a more loosely aggregated matrix in the abnormal dentin.

A series of rabbits, guinea pigs, and monkeys were subjected to varying quantities of air abrasive powder by Kerr. Some animals were subjected to air abrasive powder containing tooth dust. Some of the animals have been sacrificed immediately following a long period of exposure; others have had a period of 30 or more days without exposure to determine whether the changes will resolve or whether there is residual effect following severe exposure. Microscopic studies show marked changes in lungs in animals sacrificed immediately following exposure. The changes are more severe in the animals with the longer exposures. In animals sacrificed 30 days after last exposure, changes are residual.

A survey of 2,929 male patients has been accomplished by Kruger. All significant oral lesions were studied both clinically and histologically, emphasis being placed on leukoplakic and keratotic lesions. The presence of dyskeratosis was considered essential for the histologic diagnosis of leukoplakia, and the criteria employed for the clinical diagnosis of this disease were those commonly used in dentistry today. There were 57 lesions of all types; 24 were of a keratotic nature. Nineteen of these lesions were clinically diagnosed as leukoplakia but confirmed by histological study in only 1 instance. The other keratotic lesions were given the microscopic diagnosis of focal keratosis, focal keratosis and acanthosis, verrucous acanthosis and squamous cell carcinoma. Of all the 57 lesions disclosed, the fibroma appeared most commonly, totaling 15 in number.

Mann has conducted experimental work on the use of fluid turbine power in dentistry. Five electric drive handpieces have been constructed and delivered for testing by the U. S. Army, Office of the Surgeon General. Three turbine drive handpieces have been completed. One of these handpieces has been equipped with pressure outlets for the measurement, by means of manometer banks, of the air pressure on both sides of the nozzle plate. Everything is in readiness to determine experimentally under which conditions of operation and details of construction the turbine type dental handpiece will produce the greatest torque and speed with the air pressure available.

Studies by Schour on traumatic and chemical irritants on teeth indicate that following cavity preparation the pulp of the dogs showed a mild inflammatory reaction beginning as an infiltration of acute inflammatory cells and progressively changing into a chronic stage. At 21 days apparent recovery had occurred. At about 16 days a secondary dentin had formed, averaging about 60 microns in width. The secondary formation of dentin was more prominent in the dog than in man. In the monkey the pulpal response started as a mild acute inflammation, became chronic, and was still apparent at 26 days. Secondary dentin was 1st seen in the 19-day-old cavities and ranged in width from 30 to 120 microns. The pulpal and dentinal responses in the dog and monkey were milder than in man, with the monkey more nearly approaching the human series. The general pattern of reaction in both animals supported the conclusions drawn from the study of human material which are 1) In view of the presence of inflammation, 1st acute and later chronic in nature, base plate gutta-percha is not as innocuous a dental filling material as has been assumed; 2) There are no significant differences between the reactions of dentin and pulp in cavities prepared by the bur technique and by airbrasive technique.

Schour developed a procedure which employs the lower incisor of the rat as a biologic test object for demonstrating the early effects of local mechanical and chemical injuries of the cytoplasmic extension upon the perikaryon of the odontoblasts and upon the connective tissue of the pulp. This will be a useful biologic screening test.

The odontoblasts showed functional and morphologic alterations. The mildest functional changes were reflected in all cases in the calciotraumatic response of the dentin. The morphologic changes, which were seen in a varying number of odontoblasts, ranged from reversible albuminoid degen-

same period. 1% paraformaldehyde in deep cavities caused injuries which were still present 96 hours after the experiment.

The studies by Van Huysen on traumatic and chemical irritants verify previous findings and fundamental conceptions with respect to the vitality and recuperative powers of the healthy pulp. If the healthy pulp is capped immediately with a nonirritating material it will survive as a normal functioning organ. He has shown that satisfactory capping may or may not be followed by closure of the exposure with a dentin bridge. It is therefore suggested that if a pulp is successfully capped, the exposure area should not be reopened. There can be no future for a pulp that has been exposed for more than 6 to 8 hours, whether it be due to caries, fracture, or any other cause. Inflammation of the pulp, whether it be hyperemia or necrosis, is not conducive to successful pulp capping. Of great importance are accurate diagnosis, immediate protection, and non-irritating protective materials.

The study of teeth by Wainwright from patients treated with I^{131} indicates that the in-vitro observation of enamel surface uptake, penetration, of enamel lamellae and damaged dental structures, and the diffuse penetration of intact enamel by certain small organic molecules have all been duplicated in the living human subject in tracer studies with radioactive iodine. No difference in amount of iodine localized in the teeth could be detected by radioautographic techniques, between teeth extracted at periods after radioactive iodine administration varying from 5 hours to 19 hours. For unexplained reasons much of the experimental evidence obtained suggests that the iodine is retained in approximately the original amounts in human teeth under living conditions for atleast 2 weeks.

Wainwright has found that the diffuse penetration of I^{131} through the tooth under in-vivo conditions may be confirmed by in-vitro observations. A prominent difference between the in-vivo and the laboratory experiments appears after 2 weeks' immersion in water. In the test tube the iodine becomes more uniformly distributed through the dentin of the tooth and tends to leave the middle portions of the enamel. In the living human subjects on the other hand, several specimens have shown that localized deposits of I^{131} have been maintained for periods as long as 19 days.

Based upon studies by Weinmann of benign and malignant oral lesions, it has been determined that large amounts of phosphamidase are present in all types of malignant growths studied. Phosphamidase is present also in some types of growths commonly considered as benign, e.g. in giant cell tumors, and is absent in hyperkeratotic lesions. This may become an applicable diagnostic aid.

f. Future Plans: Studies will continue as described above.

g. References: Progress reports of investigators.

Project No. 6-63-01-06
Contract No. MD-148

NOT FOR PUBLICATION
Report Date 30 June 1952

Dental Caries

Dr. William Wainwright
College of Dentistry
University of Illinois
Chicago, Ill.

BRIEF. The investigator is conducting an autographic determination of the ability of various bacteria and other substances involved in producing the carious lesion to penetrate dental structures and filling materials by the use of radioactive tracers.

BACKGROUND. Three teeth from patients who have received Au^{198} have been cut into 16 ground sections and 28 radioautographs were prepared. The results with Au^{198} were negative, confirming the clinical observation that Au^{198} given intraperitoneally in the colloidal form does not leave the peritoneal cavity. The blood was also negative.

In-vivo human examinations of cementum metabolism are easily made on teeth from patients treated with radioactive iodine for thyroid disease. Cellular and acellular cementum were often diffusely penetrated by I^{131}. Complete penetration occurred in teeth exposed to I^{131} in-vivo in as short a time as 5 hours and was still present in teeth exposed to I^{131} as long as 18 hours. Presumably the time limits are much wider. Exposed cervical dentin was penetrated deeply to the pulp chamber. Deep penetration occurred through secondary dentin as well.

Twenty-five human teeth from such patients were sectioned, 158 ground sections made, and 410 radioautographs were made of these sections.

PROGRESS. In-Vitro Observations with Dyes - Freshly Extracted Human Teeth Stained with Silver Nitrate. Thirty-two teeth were stained by immersion in 40% $AgNO_3$ solution for periods of from 5 minutes to 30 days. One hundred and seventy-six ground sections have been prepared. Only visual observations of the extent of penetration have been made at this time.

In-vitro and In-vivo Observations with Labelled Compounds: Human In-vivo Studies of the Penetration of Teeth by I^{131}. Further conclusions resulting from the study of teeth from patients treated with I^{131} are:

The in-vitro observation of enamel surface uptake, penetration of enamel lamellae and damaged dental structures, and the diffuse penetration of intact enamel by certain small organic molecules have all been duplicated in the living human subject in tracer studies with radioactive iodine.

No difference in amount of iodine localized in the teeth could be detected by radioautographic techniques, between teeth extracted at periods after radioactive iodine administration varying from 5 hours to 19 hours.

For unexplained reasons much of the experimental evidence obtained suggests that the iodine is retained in approximately the original amounts in human teeth under living

conditions for at least 2 weeks.

The unusually large number of teeth available for this investigation was supplied by the staff of the Veterans Administration Hospital, Hines, Illinois.

In-vitro Studies of the Penetration of Freshly Extracted Human Teeth by I^{131}. In order to make possible comparison of in-vitro with in-vivo observations 15 teeth have been cut into 94 sections and 214 radioautographs made from these. Conclusions: The diffuse penetration of I^{131} through the tooth under in-vivo conditions has been confirmed by in-vitro observations. A prominent difference between the in-vivo and the laboratory experiments appears after 2 weeks' immersion in water. In the test tube the iodine becomes more uniformly distributed through the dentin of the tooth and tends to leave the middle portions of the enamel. In the living human subjects, on the other hand, several specimens have shown that localized deposits of I^{131} have been maintained for periods as long as 19 days.

Hamster Colony. The hamster colony now consists of 6 adult females, 2 adult males, and 28 offspring 20-25 days old. The 6 adult females and 28 offspring, then aged 10 days, were placed on the cariogenic diet described by W. G. Shafer (Science, 110: 143, 1949). A 2nd group of weanling hamsters was procured and placed on the cariogenic diet at the age of 25 days. It is anticipated that during the summer there will be an ample supply of in-vivo carious lesions for the experiments with S^{35}-thiourea and C^{14}-sucrose.

FUTURE. In-vitro observations of silver nitrate penetration will be continued with an attempt to clear sections in aniline oil in order to check the extent of penetration of silver nitrate observed visually by a more accurate microscopic observation. Microscopic observation is difficult at present, due to the thinness of the specimens and the presence of extraneous stains in carious areas.

The in-vitro observation of methylene blue penetration will be studied with the aid of cutting coolants permitting thin sectioning without water.

The in-vitro experiments on I^{131} penetration will be continued and manuscripts prepared describing the results.

Further investigations will be made of the conditions necessary for obtaining thin sections without the necessity of using water as the cutting coolant.

The studies of penetration by S^{35}-thiourea and C^{14}-sucrose will be continued as cutting methods are developed. Considerable progress may be possible with observations on the carious lesion of the hamster, since hamster teeth cut more easily than do human teeth.