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MEDICAL DEPARTMENT

RESEARCH PROGRAM

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1 APRIL – 30 JUNE 1949

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

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JRDB 1002

PROJECT TITLE: Prevention of Growth of Hair in Skin Grafts; Irradiation

PROJECT DESIGNATION: 6-59-08-01 CONTRACTOR AND/OR LABORATORY: U. Pa.
MD 365 (Pendergrass)
COGNIZANT AGENCY: Med Res & Dev Bd, SGO DATE: 1 Apr - 30 June '49

PURPOSE: To discover a radiation dosage that will safely remove hair from skin grafts without impairing the integrity of the graft, with the hope that this will facilitate the use of scalp skin to repair defects of the face; also, other studies on irradiation.

Animals and Equipment. The population of 250 rats was maintained during this period. Early in the quarter some of the animals developed a pneumonia but at present the colony is free of it. Other animals have been found to have a parasitic infection of the skin; the same infection has been found in the colonies at the Wistar Institute. Small mites 0.2 mm. long, not yet identified, have been removed from the hair in the submental region and so far have caused no systemic effects.

During the hot weather the air- and temperature-conditioning apparatus has given some trouble but it has been rapidly repaired.

Experiments in depilation with the long wave-length, high-intensity radiation produced by the Bracke-Seib apparatus are in progress. The air roentgens required for depilation for different qualities of radiation are being determined.

Experiments in Progress or Completed. Approximately 100 rats have been exposed to whole-body irradiation after receiving pitressin.

The following four experiments have just been completed. Male Wistar rats of 180-210 gm. weight were irradiated with 900 r whole body, 50 cm. distance, 200 KV, 0.5 mm. Cu. and 1 mm. Al. filter, at about 80r/minute. Each of the four experiments was done at weekly intervals, as 24 animals per week were received from the Wistar colony. Pitressin in aqueous solution 2 U was given intraperitoneally 30 minutes before irradiation.

Experiment No.	Condition	No. Rats	No. Rats Dead
27 A	Pitressin	12	2
B	Control	12	3
28 A	Pitressin	12	9
B	Control	12	9
29 A	Pitressin	12	3
B	Control	12	5
31 A	Pitressin	12	9
B	Control	12	8

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During these experiments an epidemic of pneumonia occurred in the colony (there was also an epidemic at the Wistar Institute at the same time).

No conclusions have yet been reached as to the protective effect of pitressin in whole-body irradiation. Instead of the small groups used here, a large group of animals should be treated with pitressin and irradiation and results compared with those in a control group.

To date, 235 autopsies have been completed on animals receiving whole-body irradiation and irradiation to the hind leg. Approximately 1100 slides have been prepared but no definite conclusions have been drawn. Principal finding was that no change was noted pathologically until nine days after exposure to x-rays. The peak of exposure was found in 16 days, but the subsidence of the reaction may not take place for some days and may be very prolonged. The hair follicles and their glands are fewer than normal, although it was not possible to make a definite count. The sebaceous glands are more affected than are the other skin structures (this was true except on the foot pad). This report is on the animals that received no protection from the pitressin. In the animals treated with pitressin before the use of radiation, the reaction is much less definite, but is present in comparison with the controls. The glands are scarce and it is impossible to compare the actual number of glands that remain with those of the control group.

Letters were prepared and sent to 90 human volunteers treated on the thigh in 1942-1943. Replies have so far been received from 17. Seven individuals have been re-examined and photographs taken of the irradiated area. Nine people have given permission for the complete excision of the area, which has already been accomplished in one case.

The theory is held that part of the local reaction due to irradiation is caused by an antibody-antigen reaction resulting from a foreign protein produced by the radiation. It has been known for a number of years that whole-body radiation interferes with antigen-antibody reaction; therefore it was concluded that if the above theory were true, local radiation following whole-body radiation should show a diminished reaction. Whole-body irradiation was given at various

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As part of the continuation of whole-body irradiation with heavy doses, a group of rats was given total-body irradiation with 1200r (air). They began to die around the fourth day; none has survived. These data are being added to previous data for various dosages.

When the hind legs of rats were iced both before and during the irradiation, some protection was noted. This is similar to the effect of pitressin and is part of those experiments. Three other vasoconstrictors were tested and they all showed a slight protective effect.

Proposed Experiments. Three other preparations of pitressin are to be tested after whole-body radiation followed by radiation of one leg, as was done in the first experiment with pitressin. This is to determine whether the effect of pitressin (in decreasing the irradiation reaction) is due to the pitressin alone or to some impurity in the preparation.

To show the effect of pitressin on arterioles both before and after its use in the rat's limbs, techniques with the use of thorotrast are being worked out; an attempt is being made to use the Bracke-Seib apparatus to take the x-ray films.

The study of the relationship of the age of the rat and whole-body irradiation is being continued.

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JRDB 1002

PROJECT TITLE: Surgery Required for Treatment of Radiation and Thermal Burns

PROJECT DESIGNATION: 6-59-08-04 MD 411 CONTRACTOR AND/OR LABORATORY: Med College of Virginia (Evans)

COGNIZANT AGENCY: Med Res & Dev Bd, SGO DATE: 1 Apr - 30 June 49

PURPOSE: To investigate the surgical procedures required to handle casualties that have been subjected to radiation and thermal burns such as those that may result from exposure to large amounts of radioactive material; and to conduct these studies under conditions and effects of cold.

The following memorandums to Dr. Evans from the members of his research staff comprise the report of progress for this quarter.

Dr. Ham (Physics Division). The first objective has been to acquire personnel, laboratory facilities, and equipment. This Division now comprises a small but well-equipped laboratory and machine shop, and an x-ray concrete structure housing a 1000-KVP x-ray machine. The major accomplishment to date has been the construction of a mass spectrometer, which is nearly complete and will be ready for initial testing in June.

All areas outside the actual x-ray enclosure have been monitored carefully with radiation instruments to assure adequate protection of personnel. The x-ray beam reflected through the beryllium window has been plotted in the vertical and horizontal plane. Absorption curves and half-value layers have been determined in lead, copper, and aluminum. A lead shutter operated by electromagnet allows exposures from two seconds to any desired length of time. All radiation measurements have been made with Victoreen r-chambers of red bakelite, black nylon, beryllium, and polyethylene. The 25-r standard chamber and one 250-r black-nylon chamber have been calibrated at the National Bureau of Standards on a 200-KV x-ray beam filtered through 1.5 mm. of beryllium.

The mixture of soft and hard x-rays makes the calibration of the 1000-KVP beam difficult, if not impossible, by ordinary means such as standard r-chambers. Present data obtained in this laboratory indicate that a considerable amount of soft radiation is present at distances closer than 100 cm. to the source. Half-value layers are a function of the type of chamber used and the distance at which the measurement is made.

Whole-body irradiation of dogs has been performed at 160 cm. from the source through 1 mm. of aluminum filter. At this distance the radiation field is uniform within a few r over the area occupied by the animal. All r-chambers agree among themselves within experimental error at this distance. To date, three dogs have been irradiated and studied.

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A masonite phantom has been constructed, and measurements are now in progress on depth dosages. Large dosages of radiation have been given selected specimens furnished by the Biology Department of the School of Pharmacy.

An air-conductivity meter is under construction. It is hoped that tests will show the value of this instrument in monitoring laboratories exposed to radioactive CO_2. The primary purpose here is health physics, but future tests may make it possible to obtain quantitative data on radioactive CO_2 exhaled from the lungs of dogs which have been injected with radioactive carbon.

The laboratory has acquired recently an AEG-50T soft x-ray tube. A power source of 50 KV at 35 m.a. must be constructed for this tube, and possibly the necessary equipment will be provided by loan or gift from a well-known manufacturer of x-ray equipment. The tube will be used for radiation-chemistry studies in conjunction with the mass spectrometer and will also be applied to studies of radiation injury in the present program on thermal-injury research.

Since r-meters are not considered satisfactory for the measurement of dosages from beryllium-window x-ray tubes, an attempt will be made to measure the temperature-rise in water due to the absorption of x-rays. Present plans contemplate the use of sensitive thermo-elements such as bismuth-tin bismuth. A sensitive potentiometer bridge is scheduled for delivery in July.

A proposed research program in the radiation chemistry of organic substances in aqueous solution has been submitted to The Surgeon General's Office. Here, it is planned to scan and analyze by means of the mass spectrometer the gases evolved from solutions subjected to intense irradiation with soft x-rays.

Dr. James (Hematology Division). The Hematology Laboratory is set up for studies of three basic types: routine hematologic determinations for general study, including hemoglobin, red and white cell counts, differential, reticulocyte counts, hematocrits, indices, fragility, etc.; biochemical determinations for the study of the blood pigments, i.e., bilirubin, urine and fecal urobilinogen, urine coproporphyrin, red-cell protoporphyrin, and serum iron and copper; and a miscellaneous group of examinations on the urine and feces which aid in the evaluation

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of various hematologic and hepatic disorders—i.e., bromsulfonthalein, plasma cholinesterase, etc.

During the past year, interest in the fate of hemoglobin in the severely-burned person was prompted by clinical observation that anemia may rapidly become profound. While undertaking this study with the use of whole-blood volumes [by P^{32} and T-1824 (Dr. Nachman)] and total circulating hemoglobin data, we became aware that all the severely-burned patients were showing a marked increase in the excretion of the urinary urobilinogen very shortly after their burn and that extremely high excretions preceded their death. The fact that they have also excreted large quantities of fecal urobilinogen has been interpreted as being due to increased destruction of blood, both their own and that infused. The data on urinary urobilinogen led us to believe that the liver in some manner may be involved early in burns, either from a "toxic" change arising at the site of the burn or perhaps from other factors. From other liver-function studies done on a few of the burn-patients, some evidence has been obtained to suggest early impairment of at least excretory function of the liver, and possibly protein-synthesis impairment. Preliminary estimations of urinary coproporphyrin excretion seem to indicate that this substance is also increased early in the severely-burned patient. Studies are in progress to determine the isomer type and concentration of the various coproporphyrins.

We hope to carry out with the Physics and Biochemistry Divisions of this project studies on the distribution of N^{15} in man and animals in the heme derivatives. Present ideas are to study the excretion of bilirubin in animals and the fate of the various heme pigments found in the stool. During the summer we hope to carry out studies on the electrophoretic mobility of hemoglobin prepared by the Biochemistry Division.

Some additions to the general function of the laboratory should be made for obtaining specimens in a satisfactory manner. This would entail setting up a metabolic ward where it would be possible to get accurate and complete urine and fecal specimens during the periods the patients are hospitalized. In addition, the metabolic ward would allow a more accurate determination of the nitrogen metabolism of patients and, more important, generally would bring us into closer observation of the clinical manifestations of burns, particularly in relation to the alteration observed in the laboratory.

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Dr. Robinett (Surgery Division). The purpose of the work with the Tiselius apparatus has been to demonstrate electrophoretic changes in the blood-protein pattern in individuals who have suffered thermal or irradiation damage.

Therefore, 12 of the patients who entered the hospital with "fresh" burns involving more than 10 per cent of the surface-area of the body were studied by this procedure. A sample taken as soon after hospitalization as possible was used as a rough control over later samples. These patients varied in age from four to 84 years, and their burns affected from 10 per cent to 98 per cent of the surface of the body. Thirty-five samples were taken from these patients, but since several died in 24 to 48 hours only one sample is present for these cases.

Gross changes in electrophoretic pattern were found in all the patients, the amount of change being proportional to the extent of the burn. These changes consisted of an increase in alpha globulin and a decrease in albumin concentration. When infection became a factor, the gamma globulin increased.

The influence of therapy on the pattern is well shown in one case where samples were taken before and after administration of albumin (100 gm.). This caused the albumin-peak to resume its former prominence.

In two patients with extensive burns, a new component moving near the gamma globulins appeared. This may be associated with a hyperfibrinogenemia, but the mobility of the peak is not characteristic of the latter substance.

Two samples of blister-fluid run with their corresponding plasmas show the preponderance of albumin in this exudate.

Electrophoretic studies of burned dogs have not been done to any extent. When one dog given a minimal burn was studied, an increase in alpha globulin was found to be associated with a decrease in albumin. Studies with electrophoretic technic have failed to show changes in the pattern following massive, intermittent doses of irradiation on carcinoma-of-the-cervix cases. Twelve cases studied prior to and several times after irradiation failed to show significant changes following their treatment.

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Dogs and rats have been subjected to irradiation and electrophoretic analyses made of their sera. Even though the animals received an apparently lethal dose of irradiation, no gross changes were found in the electrophoretic pattern in the few animals studied. Other workers on the Manhattan Project found marked changes in the alpha globulins in dogs dying of irradiation sickness; apparently dosages were much greater than the ones we use.

After a study of the literature it was found that no satisfactory standard thermal burn had been devised which could be used by any laboratory. While several workers have studied the mortality and blood-changes in animals given burns of various depths, none has tried to produce a standard procedure by which a known percentage of body-surface can be given a first-, second-, or third-degree burn. Studies are in progress on the effect of a 20-per-cent deep second-degree body-burn on a dog. If a standard bodily response to this procedure is obtained it will be of value in future studies of hemoglobin-metabolism.

The same scarcity of knowledge is found concerning a standard x-irradiated dog. It has been our desire to produce a standard response in non-lethally-irradiated animals. For this purpose we have used the 1000-KV x-ray machine and have given 250 to 270 roentgens of whole-body irradiation to three animals. The hemograms of these dogs have been very interesting and somewhat uniform.

It is proposed to continue the work in progress on the electrophoresis of sera of burned patients and irradiated animals. It is our intention to produce a standard response in a non-lethally-irradiated animal so that this animal can serve as a future research tool in hemoglobin-studies. Work on a standard thermal burn in the dog is being done for the same reason, since no adequate studies of therapy can be begun without controlled studies of normal, non-treated dogs.

Dr. Haynes (Surgery Division). The work of the Surgery Division during the past few months has included several facets. Studies on hemoglobin-degradation in the burned patient have been reported by Dr. Purnell; P^{32} red-cell-volume evaluations by Dr. Nachman; and studies of the blood-protein electrophoretic pattern and blood-volume alterations in the burned and irradiated animal by Dr. Robinett.

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In order that this and other divisional work might function well as a whole, we have actively endeavored to advance our research facilities, particularly facilities for animal experimentation. The animal laboratory unit now consists of (1) a fully-equipped operating room, (2) a treatment room convertible to a second surgery, (3) a diet kitchen, and (4) one animal-study room which will house approximately 20 animals under metabolic conditions. In anticipation of the use of carbon 14 in the animals and its excretions in the carbon dioxide, adequate ventilating facilities have been provided, with a wide margin of safety.

Fundamental observations of Schoenheimer, Rittenberg, and his group suggest that (1) the body protein pool is not static but is in a dynamic equilibrium, (2) glycine is a precursor of hemoglobin, and (3) hemoglobin in the red cell is essentially unchanged until the cell-wall is destroyed. These conclusions were gained by feeding tagged materials such as glycine to animals and man and following the buildup of the isotopic material in the red cell and serum protein, and following the disappearance of tagged transfused cells.

In the burned patient the problems of altered synthesis and degradation of hemoglobin are striking. Anemia must be constantly guarded against, and it is a common clinical observation that transfusions frequently do not produce the rise in hemoglobin which might reasonably be expected. Many factors are doubtless in operation, and the subject lacks clarity.

Several modes of attack present themselves. Information relative to the time required and the amount of hemoglobin synthesized from isotopic glycine would be of considerable interest. Further observations on the fate of the transfused red cell could be gained by following the degradation of tagged hemoglobin and its conversion to bilirubin. With these problems in mind we have constructed bile fistulae in dogs in order to obtain all the bile excreted. In this preparation we plan to transfuse tagged red cells from a donor-animal and follow the breakdown to bilirubin under normal conditions, in the severely-burned animal, and in the irradiated animal. These observations could be extended to include excretion-studies of the isolated bilirubin and further degradation-studies of bilirubin admitted to the gastro-intestinal tract and converted to urobilinogen.

We feel that considerable progress has been made, and we anticipate a continued active program of endeavor.

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Dr. Purnell. The number of burned patients hospitalized and therefore candidates for study is 51 in the seven months from 1 October 1948 through 31 May 1949. The size of the burns varies from 1½ to 94 per cent of the body-surface. Patients vary in age from eight months to 84 years, and are both white and colored. Patients with small, deep burns were frequently hospitalized after the first re-dressing. Examples of certain complicating factors which occasionally forced admission to the hospital for small burns are: associated disease such as scarlet fever and severe upper-respiratory disease; damage to the pulmonary tract from the burn; severe burns of the eye; infection in the site of the burn; carcinoma in old burn-scars; keloid formation in burn-scars; and burns in critical places (as across joint-surfaces).

In the same seven months, about 450 burned patients were treated in the emergency rooms and clinic. None of these burns was greater in area than 10 per cent of the body-surface. Burning agents included fire, water, grease, metals, electricity, sun, acids, and alkalies. No x-ray burns were seen. Reasons for non-admission to hospital included insufficient size or depth of burn, cleanliness of burned area, aid of the penicillin clinic, and lack of a complicating disease.

After the burned part was carefully inspected, therapy as follows was carried out:

Locally:

Minimal debridement.
Vaseline gauze immediately on the wound.
Pressure dressing made of padding and ace bandage.
Splinting of a part if necessary.

Systemically (depending on extent of burn and condition of patient):

Intravenous fluids, usually by cannula.
Bladder catheterization.
Tube-feeding for forced dietary intake.
Anti-tetanus serum or tetanus toxoid.
Penicillin and/or streptomycin.
Analgesics and soporific.
Stryker orthopedic frame.
"Therm-o-rite" apparatus.

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Sub-acute and chronic treatment carried out in the hospital and in the clinic followed a somewhat similar outline, attention being paid to both local-lesion and systemic status.

Long-term follow-up reviews and reconstructive surgery are being carried out, and an expansion of the present activities is planned.

Investigative work has included the problem of the hemolytic destruction of blood. The statement that not more than 10 per cent of the total circulating red-cell mass is destroyed immediately at the site of the burn has been based on blood-volume studies plus the amount of circulating

air-conditioning and filtering apparatus attached. The capacity should be either four or eight beds, available to both white and colored patients.

Dr. Nachman (Isotopes Division). This report is a review of the major program carried out by this Division, and it deals chiefly with a comparative study of red-cell volumes in human subjects as determined with P^{32} tagged red cells and T-1824 dye.

The immediate objective of this study was to develop a simple, rapid, accurate, and easily-reproducible method for measuring the red-cell volume directly. At the same time, we sought to compare the results of the P^{32} technique with the T-1824 dye hematocrit method, which measures red-cell volume indirectly.

Realization of the immediate objective would provide us with an excellent investigative tool directly applicable to the broader program of investigation of hemoglobin metabolism in irradiation and/or thermal injury.

The technique, briefly, is a modification of the one proposed by Hevesy and Nylin. The red-cell volume is determined by isotope dilution by use of the subject's red cells tagged with P^{32} *in vitro*. The details of the technique are described in a report soon to be submitted for publication.

Forty determinations were performed on 36 subjects from the surgical services of the Medical College of Virginia Hospitals. The T-1824 dye hematocrit method gave a mean red-cell volume of 2320 ± 79 cc.; with the P^{32} method the mean red-cell volume was 1850 ± 66 cc. These two mean values are significantly different (d = 470 ± 102 cc.; t = 4.6, $P < .01$). In every case, the red-cell volume as determined by the P^{32} method was less than the comparable value obtained with T-1824. The mean ratio of the $\frac{\text{RCV by } P^{32}}{\text{RCV by T-1824}}$ was 0.800 - 0.153. None of the individual ratios fell outside the range of the mean ratio ± 2½ times its standard error.

Correction of the hematocrit for trapped plasma in calculating the red-cell volume by the T-1824 method was purposely eliminated in this study. If the hematocrit is corrected by 0.95 for trapped plasma, the red-cell volume by T-1824 becomes correspondingly higher. Re-calculation of the ratio of the $\frac{\text{RCV by } P^{32}}{\text{RCV by T-1824}}$ yields a value of 0.867.
using corrected hematocrit

Similar studies of simultaneously determining red-cell volume with tagged cells and plasma volume with T-1824 dye have been conducted by

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Gibson and Meneely using radioiron and by Reeve and Mayerson using P^{32}.

The following table gives the mean values of the aforementioned studies for the ratio between RCV as determined by the isotope technique and RCV by the dye method.

Investigator	No. of Cases	Isotope	Mean Ratio RCV by isotope / RCV by dye	Hematocrit Correction Factor
Gibson	40	$Fe^{55,59}$	0.845	None
Meneely	28	Fe^{59}	0.809	None
Reeve	13	P^{32}	0.870	0.95
Mayerson, Lyons, et. al.	26	P^{32}	0.960	0.915
Present Study	40	P^{32}	0.800	None
			0.867	0.96

Excepting the value of 0.96 obtained by Mayerson et. al., there is no significant difference in the mean values above. This indicates (to me, at least) virtual confirmation of the radioiron and radiophosphorous techniques for determining red-cell volume.

We have used the P^{32} technique to measure red-cell volume immediately before and after transfusion of a known volume of red cells in a few cases. The group is not large enough to allow definite conclusions to be drawn, but the results are encouraging. In addition, we have utilized the P^{32} technique to follow the red-cell volume of two severely-burned patients in conjunction with Drs. James and Purnell.

Dr. McKennis (Biochemistry Division). In order to obtain information on the production and destruction of hemoglobin in subjects which have been injured by ionizing or thermal radiation, it was decided to study the uptake of N^{15} glycine in burned patients at the Medical College of Virginia. Previous work of Rittenberg and his associates gives information on the appearance of N^{15} in hemin chloride following ingestion of N^{15} glycine. Data were obtained in normal subjects as well as in subjects with pernicious and sickle-cell anemia. The rate of uptake and disappearance of N^{15} in heme, and other data, enabled these workers to make interpretations concerning the life of red cells and hemoglobin.

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The frequent complaint of those concerned with the surgical treatment of burns is that hemoglobin seems to "disappear" after transfusion. It has been stated (Evans - private communication) that there are no studies on the rate of synthesis and destruction of hemoglobin in such patients. Therefore, in order that N^{15} glycine might be used in the study of hemoglobin-metabolism in these patients, N^{15} potassium phthalimid was reacted with ethyl chloracetate according to previously-published methods. The resulting ethyl phthalimidoacetate will be hydrolyzed to give N^{15} glycine. Two different types of experiments are contemplated: (1) the feeding of N^{15} glycine to selected burned patients, and (2) the transfusion of N^{15} labeled red cells to normal subjects and burned patients.

In the first experiment, N^{15} glycine will be administered in divided doses to selected burned patients. At intervals of approximately three days, samples of blood will be taken and the abundance of N^{15} determined in total blood proteins, hemoglobin, and heme. It is anticipated that each experiment will extend over a period of 100 days. In order to insure that the hemoglobin is pure, it is planned to subject crystalline samples to electrophoretic analysis. Hemin chloride obtained from the pure hemoglobin* can then be compared with hemin chloride from whole blood. It is also desired in a number of instances to purify both substances to the point of constant isotopic composition. N^{15}-analyses will be made on nitrogen gas prepared from Kjeldahl digests by the action of hypobromite or nitrogen gas prepared by direct combustion of the samples.

In the event that synthesis of hemoglobin in the burned patient differs dramatically from that in the normal patient, further investigation will be warranted. It is thought that, in any event, the investigation of possible in-vitro synthesis (London and Rittenberg) of heme by blood samples from our subjects incubated with N^{15} glycine should prove interesting.

Since acetate has been shown by others to be a precursor of heme in the rat, it will be of interest to study with heavy carbon the ability of

*Miscellaneous studies on the chemical composition of "pure" hemoglobin will be carried out in conjunction with these studies. Much has been accumulating in the literature to indicate definite chemical and physical differences in hemoglobins which depend upon treatment of samples and the physiologic status of the organism. The recently-reported differences between electrophoretic mobility of hemoglobin from patients with sickle-cell anemia and normal subjects again point to probable differences in chemical composition.

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acetate to serve as a precursor of heme in the human and perhaps to compare utilization of acetate with utilization of glycine in various physiologic states.

Labeled blood obtained from experiments in which N^{15} glycine is fed will serve not only for a study of what happens in the formation of hemoglobin but will also be a source of donor-blood. It is planned to transfuse labeled red cells to burned patients. By following N^{15} nitrogen concentration as above, for example, we should be able to detect alleged differences between the ability of normal subjects to break down hemoglobin and the capacity of burned patients to do the same. In order to establish some experimental correlation between old and new cells, it is planned to follow the disappearance of injected cells serologically as well as by the disappearance of N^{15}.

Corollary experiments on dogs have been started. In part, the advantages of experiments on the dog as opposed to the human are derived from the use of bile fistulae in dogs. Direct collection of bile and the fact that standard thermal and ionizing irradiation doses can be given to animals more readily than to humans are obvious advantages. It is thought that the production of heme in the dog with carbon and nitrogen labels will enable us to study the position of the labeled carbons in heme, since degradation of the labeled heme can then be carried out by in-vitro or in-vivo methods. Another advantage in the use of the animal lies in the fact that radioactive carbon can be employed as an indicator. Radioactive carbon has been obtained from the Atomic Energy Commission.

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PROJECT TITLE: Effect of Capacitron Irradiation on Materials of Biologic Importance

PROJECT DESIGNATION: 6-59-08-07 CONTRACTOR AND/OR LABORATORY: MRL, Chicago

COGNIZANT AGENCY: Med Res & Dev Bd, SGO DATE: 1 Apr - 30 June 49

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PURPOSE: To determine the effect of capacitron irradiation on the preservation and nutritional properties of food and the pharmacologic effects of this irradiation on biologic materials such as vaccines.

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As was indicated in the previous report, commercial rat food, ground and packaged in paper bags, has been irradiated and its effects on the growth and reproductivity of albino rats studied. So far there have been no differences between the rates of growth of experimental and control animals.

Breeding experiments under way will be continued for at least five generations. These studies are designed to answer the question as to whether capacitronized food has any effect on fertility or on the nursing of the young.

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JRDB 1002

PROJECT TITLE: Rehabilitation of Patients with Diseases of Military Importance

PROJECT DESIGNATION: 6-60-09-01 CONTRACTOR AND/OR LABORATORY: MNL, Chicago

COGNIZANT AGENCY: Med Res & Dev Bd, SGO DATE: 1 Apr - 30 June 49

PURPOSE: To study rehabilitation of patients suffering from diseases of military importance in order to develop and elaborate methods for shortening convalescence.

Beriberi Heart Disease and Nutritional Hypertension. The basic problem was to study the genesis of the nutritional hypertension that has been reported in patients with beriberi heart disease when they are treated with thiamin, and if possible to determine whether the hypertension occurs on the basis of humoral or nervous mechanisms. None of the patients studied at the House of Correction (Bridewell), Chicago, developed hypertension when treated with thiamin; therefore, the problem remains unsolved. However, the observations reported below of a low incidence of physical signs of gross malnutrition in the population at Bridewell raises a question of great importance to the Army. A large portion of this population consists of alcoholics and tramps, types who in the past have been considered especially prone to develop pellagra, beriberi, and other syndromes of malnutrition. The important question raised by the present observations is: Has fortification of cereals and bread with synthetic vitamins prevented gross malnutrition in these people? It is believed that this is the case, and it therefore seems that many of the Army's nutritional problems in healthy troops in relation to vitamin deficiencies can be solved by an adequate fortification program.

Detailed Nutritional Studies of Men Suffering with Chronic Alcoholism. Sixteen alcoholics, selected because they had stigmata commonly attributed to malnutrition, and nine alcoholics selected as controls (having none of these stigmata), have been studied intensively. The observations included detailed dietary histories, and serial clinical studies of cardiovascular function by different techniques before, during and after therapy with thiamin, placebos or cardiac drugs. In addition, roentgenologic small-bowel patterns were observed by Weintrobe's rapid technique; fasting-hour specimens of urine were analyzed for thiamin and riboflavin; and serial determinations were made of hemoglobin and plasma protein. Preliminary conclusions based on these data are:

1. None of the men had eaten an "adequate" diet (by standards of the Food and Nutrition Board) before admission to hospital.

2. In all patients the dietary intake was "low" in consumption of leafy green and yellow vegetables, citrus fruit, milk, cheese, ice cream and butter.

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3. Cardiovascular measurements such as ECG, circulation times and venous pressure were no different for patients treated with thiamin and untreated patients.

4. The fasting-hour urinary excretion of thiamin and riboflavin (measured on admission) fell within the "normal" range.

5. Small-bowel patterns were not of value in establishing a diagnosis of nutritional deficiency in the patients. Serial observations showed alterations in small-bowel pattern toward "normal" as frequently in the untreated alcoholics as in the treated alcoholics.

6. Among the 16 alcoholics with minor stigmata on the skin and mucous membranes, there was one case of Wernicke's encephalopathy and one case of nutritive failure (polyneuritis, apathy, and fissured, red, sore tongue with papillary atrophy). These were the only cases among over 20,000 newly admitted inmates examined, who showed clear evidence of nutritional deficiency as demonstrated by dietary inadequacy, history and physical examination, low urinary vitamin excretion, and response to vitamin therapy.

Nutritional Survey of Alcoholic and Other Newly Admitted Inmates of the House of Correction. In order to obtain accurate data on the frequency of stigmata attributed to malnutrition and the physical status of a representative alcoholic population and because a careful two-year search for primary nutritional disease in the outpatient clinic of the Research and Educational Hospital had not been fruitful, a nutritional survey was started at the House of Correction in March, 1949. The techniques used were essentially those employed by Youmans, Bean, Ashe, Johnson, Kark and others in examining soldiers and civilians during the past 10 years, but were more complete in certain details (e.g., palpation of abdomen, examination of the chest). The survey was completed early in June, and the resulting statistics are being compiled. Data are available on 452 patients, mostly alcoholics, out of 980 admissions. Hemoglobin determinations were made on the blood of 380 of the men. When physical examination was suggestive of nutritional deficiency disease, a detailed dietary history was obtained from the individual under study and at the same time a detailed dietary history was taken from one of the patients in whom physical examination revealed no evidence of nutritional disturbance. Twenty-five pairs of men were studied in this manner.

In this group of 452 no case of beriberi, pellagra, scurvy, Wernicke's disease or polyneuritis was discovered. The men were, generally speaking, typical chronic alcoholics: rheumy-eyed, dirty, unshaven, thin and prematurely aged. Some were covered with "wine sores" or the pigmented scars of old septic lesions.

Distribution curves of height and weight are being prepared. Hemoglobin averaged 14.5 gm/100 ml blood, ranging from 10 gms to 19.9 gms/100 ml blood. Analyses of diet histories obtained from the 25 pairs of patients show that their food intake was the same quantitatively and qualitatively as the dietary intakes of the alcoholics who were studied intensively.

It is tentatively concluded that gross malnutrition is rarely present among chronic alcoholics living in the Chicago area. Moreover, there is little to suggest that these men are suffering from avitaminosis.

In view of the facts that pellagra, polyneuropathy, and beriberi heart disease were extremely common among chronic alcoholics in Boston, Cleveland and New York before 1941, and that the diets of the alcoholics in Chicago are similar to those consumed by the pre-war alcoholics (consisting mainly of bread, doughnuts, coffee, stews and hashes), it is suggested that the fortification of bread has protected the alcoholic from the ill effects of B-complex deficiency disease.

A detailed study is being made of the incidence of nutritional disease in hospitals from 1938 to 1948, including statistical data on food intake, economic status and incidence of nutritional disease.

JRDB 1002

PROJECT TITLE: Fluorine and Dental Caries

PROJECT DESIGNATION: 6-63-08-01 MD 470 CONTRACTOR AND/OR LABORATORY: U. Calif. (Becks)

COGNIZANT AGENCY: Med Res & Dev Bd, SGO DATE: 1 Apr - 30 June 49

PURPOSE: To determine the effects of topical application of fluorine on the incidence of caries.

The facilities of the dental clinic of the University of California, Berkeley Campus, have been placed at our disposal for this project, work on which was inaugurated in February 1949. The first pilot-study was begun 2 May, and details were worked out to handle students on a large scale when the fall semester starts in September.

In May, 80 students applying for dental service volunteered as the preliminary group. They received four appointments for the following treatments:

Dental roentgenograms (one set available to the student upon completion of the series of four treatments). Bacteriologic examination of student's saliva as an index of dental-caries activity and survey of food intake. Thorough dental prophylaxis and first application of 2-per-cent sodium fluoride.

Thorough clinical dental examination. Second application of sodium fluoride.

Third application of sodium fluoride.

Fourth application of sodium fluoride.

Upon completion of this series of four applications a second bacteriologic examination of saliva was made. This group of students will be recalled in May of 1950, 1951, and 1952 for roentgenograms, dental examination, and saliva-specimen.

The appointments were made from 6:00 to 10:00 P.M. Monday through Friday, requiring a total of three weeks to complete one series of appointments. The clinical staff consisted of four clinicians each evening, a secretary, and one laboratory helper. The latter sterilized instruments, resupplied dental trays, etc., for the next evening's operation.

When this grant was applied for it was felt that one dentist could handle the project expediently on a full-time basis. He was to be assisted by a dental hygienist for prophylactic treatment, a laboratory technician for studies on Lactobacillus acidophilus, and a secretary to maintain records and handle patients. Since it soon became apparent, however, that it would take too long to study 2,000 students on this basis, an appeal for

volunteers was made to practicing dentists in the East Bay area. Fifteen dentists and the members of the staff of the Division of Dental Medicine, College of Dentistry, were invited to participate in the clinical operations. For purposes of uniformity, one member of the latter staff was placed in charge of all roentgenographic work, and three other members of the staff handled the dental examinations.

The findings of this preliminary group are being compiled at present. Preparations are being made during the summer for the study which will begin in September.

Screening students and arranging schedules for students and volunteering dentists have been the most time-consuming phases of the study. Only those students were selected who expect to be residents of the Berkeley Campus for the next three or four years. Previously-arranged appointments had to be changed because of evening classes. In general, however, this first pilot-study worked out satisfactorily.

In September, five groups of 80 students each and an additional group of 200 will be screened. The latter will serve as replacements in cases of last-minute cancellation. The screening will be continued during registration in February 1950 for the spring semester. It is anticipated that a total of 700 to 800 students will have been treated by the close of the spring semester; i.e., May 1950. During the summer these data will be evaluated statistically and the program continued in September 1950, until 2,500 students have been studied. They will be divided into five groups:

1. 500 students receiving full-mouth 2-per-cent sodium-fluoride applications.

2. 500 students serving as a control to Group 1, receiving topical application of a similarly-colored solution containing no fluorine.

3. 500 students receiving 2-per-cent sodium-fluoride application for half the mouth, while the other half will receive an application of the non-fluorine solution.

   (These 1,500 students of Groups 1, 2, and 3 will receive all preliminary studies mentioned above, including dental roentgenograms, bacteriologic examination of saliva, food-intake survey and thorough oral prophylaxis.)

4. 500 students receiving the preliminary studies including prophylaxis, but no topical application of any solution.

5. 500 students receiving the preliminary studies, but no prophylaxis and no topical application of any solution.

It is felt that this experimental arrangement will provide adequate controls and will yield statistically-significant data which will permit definite conclusions to be drawn as to the value of topical application of sodium fluoride and prophylaxis in the control and prevention of dental caries.

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JRDB 1002

PROJECT TITLE: Cold, Study of Physiological Effects of

PROJECT DESIGNATION: 6-64-12-02 CONTRACTOR AND/OR LABORATORY: MDFRL, Ft. Knox

COGNIZANT AGENCY: Med Res & Dev Bd, SGO DATE: 1 Apr - 30 June 49

PURPOSE: To study the physiology of subjects in cold environments.

Studies of Physiological Problems under Field Conditions in Extreme Cold. The reports mentioned previously will soon be finished.

Study of the Effects of Subnormal Temperatures on Various Enzyme Systems. In a report entitled "The Influence of Certain Endocrine Secretions on Amino Acid Oxidase" data are presented relating blood amino acid levels and the secretions of the hypophysis, adrenal and thyroid to the activity of amino acid oxidase in the liver and kidney of rats. The following is an abstract of the report:

1. Administration of a casein hydrolysate to normal rats produces an increase in the amino acid oxidase activity of the liver and kidney of these animals.
2. Administration of a casein hydrolysate to adrenalectomized or hypophysectomized animals does not produce this increase in the amino acid oxidase activity of liver.
3. Adrenal cortical extract accelerates the activity of the enzyme in the liver in vitro and in vivo.
4. The pituitary-adrenal cortex system mediates the stimulus for the acceleration of amino acid oxidase activity of the liver observed after administration of amino acid.
5. The nature of the mechanism of the pituitary-adrenal cortex stimulation after administration of amino acid remains to be investigated.
6. Livers of hypophysectomized animals show increased amino acid oxidase activity which may be due to the absence of the growth factor of the pituitary.
7. Thyroidectomized animals show a decreased amino acid oxidase activity of the liver but an increased activity of the kidney. Administration of amino acids stimulates the liver oxidase.
8. Epinephrine inhibits amino acid oxidase activity of the liver. The effect of insulin is doubtful under our experimental conditions.
9. The amino acid oxidase activity of the liver and the kidney may respond similarly to certain endocrine stimuli. However, it appears that the blood amino acid level may influence the oxidase of the kidney directly but not that of the liver.

Study of the Effects of Hypothermia on the Control of Endocrine Secretion. A report on one phase of this work is in preparation and will appear under the title "Effect of Hypothermia on the Adrenal Cholesterol Content of Rats."

The changes in the total adrenal cholesterol manifest themselves mainly in the ester fraction. Apparently male rats are more resistant to hypothermia than females. The degree of loss in adrenal cholesterol is dependent upon the weight of the animal. Administration of aqueous adrenal cortical extracts inhibits the fall of adrenal cholesterol observed in rats exposed to low temperatures.

In continuation of these studies, the effect of hypothermia on the adrenal cholesterol content is determined in rats which have been exposed to low temperatures for long periods of time. Experiments are also being carried out to determine whether different strains of rats will show the same degree of resistance to cold.

Study of Thyroid Function at Low Environmental Temperatures with the Aid of Radioactive Iodine. A report on one phase of this work has been submitted as "The Response of the Thyroid Gland to a Low Environmental Temperature as Studied with Radioiodine." An abstract follows:

> In order to determine the effects on thyroid function of varying amounts of exposure to cold, studies were carried out on rats, with the uptake of radioiodine used as a measure of the functional activity of the gland.
>
> The studies of Leblond et al were extended to shorter and longer time intervals of exposure to cold. Male rats were exposed to $4^{\circ} \pm 0.5^{\circ}$ C. for periods of from two hours through 60 days. A diminished amount of radioiodine was found to be present in the experimental animals at exposure-intervals under six hours. The amount present at about six hours was the same as for the controls and increased after 12 to 24 hours. The increased uptake was most pronounced at seven to nine days and gradually returned to control values by 40 days. The cause of the apparent inhibition at short intervals of exposure is as yet unknown.
>
> Studies are being carried on to give further insight into the mechanisms of the observed changes. A histologic and autoradiographic program has been included. Studies in

the changes of the fur in animals exposed to cold for long periods and studies on acute low-temperature exposures have been done. Tentative results show that there is a 20-to-25 per cent increase in fur after 60 days' exposure of rats to 4° C. Results of more acute cold stress confirm the earlier results.

Studies of the Effect of Hypothermia on Hematologic Function. Since Arctic studies revealed that hematologic changes take place in man exposed to cold, it was decided to attempt to elucidate these changes by a prolonged study of animals (rabbits) under varying degrees of hypothermic stress.

Procedures have been set up for the determination of hemoglobin, red and white-cell counts, differential white-cell count, sedimentation rate, blood specific gravity, blood volume, and hematocrit. Blood chemistry procedures will be included later. Normal values have been established by a series of 24 determinations on six normal rabbits. There have been two acute experiments of two weeks' duration each, during which three rabbits were exposed to a 15°-F. environment; complete blood studies and urinalyses were made every other day. Food consumption and water intake and output were plotted. Three rabbits left at room temperature underwent the same experimental procedures for purposes of control observations. Studies will continue on the hematologic effects of chronic exposure of rabbits for several months to low environmental temperatures. From time to time animals will be sacrificed for gross and microscopic examination of certain organs and blood-forming centers.

Studies on Control of Foot Sweat. Some preliminary data on the control of foot sweat were presented in a previous report entitled "Preliminary Observations on Physiological, Nutritional and Psychological Problems in Extreme Cold."

Considerable additional data have been collected on this problem. Further observations confirm that a barrier (rubber) sock reduces the total recoverable sweat by more than two-thirds—even up to 90 per cent. The physiologic basis for this decrease is being studied by two methods: (1) setting up a known vapor pressure by wearing a saturated sock next to the foot beneath the barrier and measuring the water transfer; (2) wearing the barrier between different layers of socks progressively outward from the foot to determine whether a linear increase in recoverable sweat occurs. The latter observations are almost completed and a linear effect appears to be fairly well established. Studies will be made on the use of the rubber sock in conjunction with anhidrotic foot powders already developed and tested.

JRDB 1002

PROJECT TITLE: Behavior of Peripheral Blood Vessels

PROJECT DESIGNATION: 6-64-12-12 MD 389 CONTRACTOR AND/OR LABORATORY: Tulane U. (Burch)

COGNIZANT AGENCY: Med Res & Dev Bd, SGO DATE: 1 Apr - 30 June 49

PURPOSE: To study the behavior of the peripheral blood vessels under tropical and cold environmental conditions, with particular reference to acclimatization and injury.

Since the previous report was submitted there have been no new developments; the program is continuing as it was outlined then.

An article entitled "Special Sample Tray for the Continuous Gas-Flow Type Counter Tube," by G. E. Burch, J. A. Cronvich, T. Ray, P. B. Reaser, and S. A. Threefoot, appeared in Science, May 20, 1949, pages 516-517.

A paper entitled "Considerations of Renal, Hepatic and Extremital Arteriovenous Differences in Concentration of Radiomercury of a Mercurial Diuretic," by J. P. Milnor, G. E. Burch, C. T. Ray, S. A. Threefoot, and G. S. Berenson, has been submitted for approval for publication.

An abstract, "Rates of Turnover and Biologic Decay of Chloride and Chloride Space in Dogs Determined with the Long-Life Isotope (Cl^{36})," by G. E. Berenson, S. A. Threefoot, and C. T. Ray, will be submitted in the near future.

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