

[REDACTED]

MEDICAL SERVICE

RESEARCH PROGRAM



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PRIORITY IC AND 2 PROJECTS
30 SEPTEMBER 1951

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

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Washington National Record Center
Office of the Army Surgeon General
Record Group 112

Accession #: A57-70 (546)

Box #: 560 2

File: Research Program 30 Sept 1951

30 SEPTEMBER 1951
Reports Control
Symbol CSGLD-346

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Allen, J. Garrett,
AMSGS

SECURITY CLASSIFICATION

PROGRESS REPORT (QUARTERLY)

RESEARCH AND DEVELOPMENT PROJECT CARD (NEW PROJECTS)		2. SEC.	8. PROJ. NO. 6-59-08-5
1. PROJECT TITLE Ionization Effects		5. REPORT DATE 30 Sep 57	
6. BASIC FIELD OR SUBJECT		7. SUB FIELD OR SUBJECT SUB GROUP	
8. COGNIZANT AGENCY	12. CONTRACTOR AND/OR LABORATORY		CONTRACT/V. O. NO.
9. DIRECTING AGENCY			
10. REQUESTING AGENCY	13. RELATED PROJECTS		17. EST. COMPL. DATES
11. PARTICIPATION AND/OR COORDINATION	14. DATE APPROVED		RES.
	15. PRIORITY		DEV.
	16.		TEST
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20. REQUIREMENT AND/OR JUSTIFICATION			
21. BRIEF OF PROJECT AND OBJECTIVE			
<p>Dr. Allen's project, formerly reported on a monthly basis, now has a 1C priority. A manuscript has been prepared for publication entitled "Blood Transfusion in Irradiation Hemorrhage; its Failure to Prevent Hemorrhage or Improve Survival Rate in the Irradiated Dog."</p> <p>The 515-page report, "Determination of the LD₅₀-LD₁₀₀ in Dogs. Effects of Whole Blood Transfusions on Survival," covered November through June 1951. From Discussion: Other laboratories reported variable LD values following total body irradiation. Here there has been a uniform cumulative mortality of 100% at 30 days in 157 mongrel dogs exposed to 450r total body x-radiation. Other investigators reported LD values of from 85% to 90-100% for animals exposed to 450r total body x-radiation. This series was designed to establish the LD₅₀-LD₁₀₀ (LD - The per cent cumulative mortality at 60 days following total body x-radiation) in dogs for this laboratory. Conditioned mongrels were exposed to 225r, 275r, 325r, and 375r total body x-radiation. Each series, except 375r animals, was divided into 2 groups: 1 received freshly citrated whole-blood transfusions amounting to 5 cc/kg plus loss for laboratory studies, and the other served as paired controls for the transfused dogs. The cumulative mortality in such series was either the same for the control and transfused groups or was actually higher in some of the latter. Over-all mortality of controls in the 225r series was 40%; in transfused animals receiving an identical exposure there was a 60% cumulative mortality. One transfused dog developed a hematoma and died from shock after a femoral arterial puncture. Considering this an accidental death, the cumulative mortality of the 225r transfusion group would be 50%. Over-all mortality for the 275r control and transfused dogs was 80%. Controls in the 325r series exhibited 92% cumulative mortality and transfused dogs, 100%. Animals receiving 375 r total body x-radiation had cumulative mortality of 100%.</p>			
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Whole-blood transfusions did prevent to a great extent, postirradiation anemia but failed to prevent the usual depression of the other formed elements of the blood. No beneficial effects were observed from transfusions in the reduction of abnormal bleeding associated with irradiation injury. From the mortality data secured in these studies it is apparent that the whole-blood transfusions alone are not beneficial to survival of dogs following total body x-radiation in the range of LD₅₀-LD₁₀₀.

The manuscript listed in the 1st paragraph above, received 1 October 1951 states: One hundred and forty-three dogs were exposed to single doses of total-body x-radiation ranging from 225 to 450r. All the dogs were mongrels, received the same pre-irradiation care and were essentially the same size. Both male and female dogs were used. Discussion. These data indicate that blood transfusion alone under conditions described is of no benefit either in preventing or treating irradiation hemorrhage or in altering the changes in coagulation reported for treated animals. In some bleeding actually increased in severity after transfusion, accompanied by further increase in whole-blood clotting time. Some of these animals displayed symptoms of transfusion reactions. Possibly others had mild reactions not clinically detectable. Transfusion reactions in the nonirradiated dog are remarkably rare, whereas after irradiation they occur with sufficient frequency to present a definite risk. The reactions noted were anaphylactoid. Alterations in the clotting time and in the protamine titration when they resulted from the transfusion reaction were overcome by the administration of protamine sulfate or toluidine blue. Bleeding from ulcerated areas, the continued formation of petechiae, and the hemorrhagic gastro-enteritis, however, were not influenced by these agents.

If man, like the dog, is more susceptible to transfusion reactions following irradiation injury when he is already bleeding from thrombocytopenia, capillary injury, and ulcerative alimentary lesions, the administration of whole blood transfusion should not be attempted unless protamine sulfate or toluidine blue are available in case transfusion reactions occur and increase the bleeding tendency./ The 1 beneficial effect of blood transfusion shown by these tests is the correction of anemia providing sufficient quantities of blood are given. If these data can be applied to man, 300 to 500 ml of blood given 3 times per week, starting on the 4th day should be sufficient to maintain the erythrocyte count above $3\frac{1}{2}$ million per cmm and hemoglobin concentrations above 10 grams per cent. It is apparent, however, that the postirradiation anemia in these dogs was of little, if any, consequence in determining their fate or rate of survival. Our observations should not discourage use of blood with other therapy in irradiation injury since these animals died before an anemia incompatible with life developed. These data do not take into account the possibility that blood transfusion combined with antibiotic and/or other therapy may prove beneficial./ This laboratory earlier observed that blood transfusion combined with daily administration of aureomycin enabled 2 of 11 dogs to survive a 450r total-body irradiation whereas 14 similarly irradiated dogs receiving aureomycin on the same schedule, but without transfusion died, suggesting that blood transfusion and aureomycin together fare better than aureomycin alone. More data are necessary. Summary. 1) Frequent whole-blood transfusion alone and without antibiotics in dogs exposed to irradiation at dosages ranging from 225 to 450r is without any benefit in protecting against hemorrhage, the prolonged clotting time, thrombocytopenia, neutropenia, the febrile state, and weight loss characteristic of the untreated postirradiation sickness. 2) Postirradiation anemia in these dogs can be controlled with frequent whole-blood transfusions. 3) The prevention of anemia by transfusion alone did not influence in any way the length or rate of survival of irradiated dogs. Conclusion. 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 irradiation (LD₅₀-LD₁₀₀). A more cautious attitude toward frequent blood transfusions alone in treatment of

the latent symptoms of irradiation injury in man may be indicated.

1 October note: This study is continuing at 175r, for at that level there should be little mortality in controls. Twenty have been exposed thus far, half for controls. To date 2 of 5 transfusion animals have died in less than 30 days, whereas 5 controls are living longer than 30 days. The remaining 5 in each group are only recently started.

AMSGS. Clinical Use of I¹³¹. Eighty-one diagnostic I¹³¹ uptakes by the thyroid gland 24 hours after oral administration of the tracer have been done in this period, bringing the total diagnostic uptakes since 1 January 1951 to 181. Results have been correlated with clinical findings and laboratory data. Gradient counts have been done on 14 patients requiring 6 to 8 counts per patient in an attempt to evaluate the rapidity of iodine uptake by the thyroid gland in different disease states thereof. Plateau calculations on the iodine-holding power of the thyroid gland have been accomplished on 20 patients, requiring 3 24-hour counts on each. A total of 126 determinations of butanol extractable thyroxine from sera of 50 patients who have received tracer doses of I¹³¹ have been accomplished. This is an attempt to learn the ability of the thyroid gland to make and secrete thyroxine. The influence of ACTH and TSH is being incorporated in selected cases. Cases studied include normals, hyperthyroids, hypothyroids, thyroiditis, and 1 patient with bilateral adrenalectomy for prostatic carcinoma. Two cases of thyroid carcinoma have been studied by these and by radioautographic techniques. They have been treated with therapeutic doses of I¹³¹. Since beginning, 300 I¹³¹ uptakes have been done.

Clinical Use of P³². P³² has been used therapeutically in 5 cases of malignant disease with widespread metastases. All were given divided doses at weekly intervals: One widespread lymphosarcoma. 2nd series of P³² therapy gave marked clinical improvement. Two cases of carcinoma of breast with evidence of CNS lesions, and in 1 case, bony metastases. Results showed a marked relief from pain in 1 case. The 2d is a repeat series on a patient who received marked benefit from the 1st series; it is too early to evaluate progress. One case of adenocarcinoma of undetermined origin with widespread metastases. Patient expired before series was complete. No improvement. One case of widespread sarcoma, undifferentiated, primary site unknown. Patient expired before completion of series. No improvement. Summary of Previous Progress. A series of breast carcinomas with widespread metastases has been accumulated, testing palliative effect of small multiple doses of P³². Six patients were included with marked relief in 3, fair relief in 1, and no relief in 2. A series of polycythemia vera is under way. All cases so treated to date are well controlled.

Effects of Antibiotics on Radiation Syndrome. Thirty-seven dogs have been irradiated in the current period. An LD₈₀ for the 250 KV maxitron in the Department of Biophysics has been established as approximately 325 air r at 250 KV with 30 Ma, using 1 mm of aluminum plus .5 mm copper added filtration. Target distance 100 cm. Comparison of dogs taken directly from the street with dogs in good condition from our animal house receiving the same air r under the same conditions showed an LD₁₀₀ for those in questionable nutritional and health states versus and LD₆₅ for dogs in good condition. A series of 19 dogs receiving 350 total body r delivered half to each side showed 100% mortality both for control dogs and those treated with aureomycin. However, the time of survival of those receiving aureomycin averaged twice as long as those receiving no treatment.

f. Future plan is to continue the comparison of controls and aureomycin-treated dogs, using our newly established LD₈₀ dose of 325 r and lower dosage levels in an attempt to arrive at a standard dosage for a larger series.

Tissue Response to Radiation. Eight pilot experiments measuring the uptake of oxygen by normal rabbit marrow slices have been completed, the linearity of respiration noted, and QO₂ (N) values calculated. Further data on in vitro respi-

ration rates and RQ's are to be collected, using normal marrow, after which any changes in respiration of the tissue after in vivo irradiation are to be investigated. Summary of previous progress: Normal water, fat, and nitrogen content of rabbit 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 Kheldahl nitrogen on all flask contents have shown an over-all check of 1.31% between identical samples and Q_{O_2} (N)'s, based on these values have shown reasonable uniformity of respiration from 1 sample to the next.

Preparation of S35 Labeled Amino Acids Biosynthetically, using Mycobacterium phlei. Further progress has been made in separating cysteine and methionine from hydrolysates of M. phlei protein. Exchange resins have been used to remove interfering amino acids. Pure crystalline methionine has been isolated from M. phlei protein.

Factors Affecting Radiation Mortality in the Neonatal Period - Influence of Weaning, Age, Weight, Sex, and Endocrine Patterns. To date 300 mice have been irradiated, divided into batches of 50 with litters of 5. Mice have been irradiated at age 1 day, 7 days, 15 days, 15 days, 30 days (male), 46 days (male), 44 days (female). All are routinely weaned at 30 days and observed for 60 days after irradiation. Present evidence indicates that postradiation mortality is maximal in the 30-day-old mice and that radiation mortality of the suckling mice does not exceed the random mortality of the control population similar in age and weight. Radiation side effects, such as growth and epilation, seem most marked if radiation is delivered when that specific function is maximal. Thus retarded hair development was seen in animals irradiated at 7 days but the 1-day animals never exhibited this phenomenon. Weights have been correlated with radiation changes.

f. Future plan: To complete accumulation of all this background data - 60-day observation and irradiation of 30-day-old females, 1-day-old mice not previously nursed and 21-day-old mice in 2 groups, 1 weaned immediately after irradiation and the other group continued at nursing until age 30 days. It is planned to treat 45-day-old mice with growth hormone, to study the milk protection given by splenectomized mothers and to attempt to establish a possible "milk factors" participation by continuing mouse-milk administration in the postweaning period.

MNL (with Dr Evans)

SECURITY CLASSIFICATION
PROGRESS REPORT (NC)

RESEARCH AND DEVELOPMENT PROJECT CARD (NEW PROJECTS)		1. SEC.	PROJ. NO. 6-59-08-06	
1. PROJECT TITLE Radiation Injury in Man and Animals		5. REPORT DATE 30 Sep 51		
6. BASIC FIELD OR SUBJECT		7. SUB FIELD OR SUBJECT SUB GROUP		
8. COGNIZANT AGENCY	12. CONTRACTOR AND/OR LABORATORY		CONTRACT/W. O. NO.	
9. DIRECTING AGENCY				
10. REQUESTING AGENCY	13. RELATED PROJECTS		17. EST. COMPL. DATES	
11. PARTICIPATION AND/OR COORDINATION	14. DATE APPROVED		RES.	
			DEV.	
			TEST	
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15. PRIORITY		16.	18. FISCAL EST'S.	
19.				
20. REQUIREMENT AND/OR JUSTIFICATION				
21. BRIEF OF PROJECT AND OBJECTIVE				
<p>The priority of this project was changed from 1B to 1C on 10 September 51.</p> <p><u>Quantitative Identification of Canine Erythrocytes.</u> The development of a serologic method, mentioned in previous reports, has been completed. The technique involves dilution of the oxalated blood sample with fresh serum containing ACD dog agglutinins, and the use of a high titer anti-A agglutinin serum from which the nonspecific hemolysin has been removed by adsorption by fresh CD red cells.</p> <p><u>Survival of Infused Red Cells in Dogs.</u> In previous studies it was shown that red cells in dogs made polycythemic by infusions are destroyed at rates of 1 to 1.8 per cent per day. Erythropoiesis is depressed in a manner analogous to that in transfusion polycythemias of normal human males (<u>Ann. Surg.</u>, 130: 723 (1949)). In current studies with dogs in which replacement transfusions with serologically identifiable cells have been performed, the survival of cells and erythropoiesis proceed at normal rates. The studies are being extended to survival of cells after radiation injuries.</p> <p><u>Metabolism after Thermal Burns and Radiation Injury.</u> Studies of the metabolic changes associated with thermal burns and radiation injury are continuing in dogs, in addition to inquiries into the pathogenesis of the metabolic abnormalities. The influence of therapeutic regimens, including dietary, hormonal, and other measures, is being investigated.</p> <p>NOTE: Effective 3 October 1951, this study will become part of 6-60-11-17, <u>Metabolism and Nutrition.</u></p>				
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RESEARCH AND DEVELOPMENT PROJECT CARD - (NEW PROJECTS)		2. SEC. U	3. PROJ. NO. *6-59-08-13
1. PROJECT TITLE Effects of Irradiation		4. *(see Block 21)	
6. BASIC FIELD OR SUBJECT		7. SUB FIELD OR SUBJECT SUB GROUP	
8. COGNIZANT AGENCY	12. CONTRACTOR AND/OR LABORATORY		CONTRACT/W. O. NO.
9. DIRECTING AGENCY			
10. REQUESTING AGENCY	13. RELATED PROJECTS		17. EST. COMPL. DATES
11. PARTICIPATION AND/OR COORDINATION	14. DATE APPROVED		RES.
	15. PRIORITY		DEV.
	16.		TEST
			OR EVAL
			F Y 18. FISCAL EST'S.
19.			
20. REQUIREMENT AND/OR JUSTIFICATION			
21. BRIEF OF PROJECT AND OBJECTIVE			
<p>NOTE: In December by action of the Medical Service Technical Committee the studies reported herein will be brought under this number. The future number is used here because the reports from the source were so presented. The former numbers appear in parentheses after each title.</p> <p><u>Early Effects of Ionizing Radiation (was 6-64-12-06-14 & -29). Effect of Protecting Substances (Glutathione, Cysteine) on Desoxyribose Nucleic Acid (DNA) Metabolism and Mitotic Activity.</u> The effect of cysteine on the inhibiting effect of x-rays (200 KV, 6 ma, $\frac{1}{8}$ mm Cu plus 1 mm Al, 880 r total/air) on DNA new formation was studied in rats, the intestinal mucosa with its high mitotic activity being used as a test organ. Cysteine protects to a certain degree the new formation of DNA against the inhibiting effects of x-rays. Characteristic findings are presented in Table 1./ Parallel studies of the mitotic activity show that there is also a profound decrease in the number of mitoses in the cells of the crypts of Lieberkuhn. In general the tissues of the cysteine-treated animals showed more mitotic figures than those of the untreated animals. <u>Early Effects of X-rays on Mouse Cornea.</u> This tissue shows high mitotic activity and is better suited to laboratory study than tadpole epidermis (Luther and Krebs) since it is available throughout the year. Preliminary experiments have been done to determine the dose-effect correlation with the primary and secondary effects characterized by "bridging" and "fragmentation." In addition to the orcein-staining method, phase contrast microscopic techniques are being used. <u>Studies with Triphenyltetrazolium Chloride (TTC).</u> Besides being a detector of radiation effects, TTC seems to be able to protect against radiation damage. TTC (up to 0.85 mg/cc) was given to mice 10 minutes before x-irradiation (620 r) and the survival time determined. Characteristic results are shown in Figure 1. The protective effect is similar to that reported for methylene blue.</p> <p>Abstract of AMRL Report 60, "Protection of Single Cells and Small Cell Groups"</p>			
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TABLE 1

DNA-CYSTEINE-IRRADIATION EXPERIMENTS

Experiment	Rat	DNA Sp. Act.	Inorganic Sp. Act.	DNA Inorganic	DNA New Formation %
NA 39	A	560	30520	1.9	100
	B	540	28440	1.9	
	C	339	31320	1.1	61
	D	502	28250	1.8	
NA 40	A	260	21250	1.2	50
	B	582	24770	2.4	
	C	329	28200	1.2	41
	D	708	24400	2.9	
NA 41	A	241	19400	1.2	55
	B	341	22600	2.2	
	C	149	18650	0.8	47
	D	487	19730	1.1	
NA 45	A	500	33850	1.5	56
	B	854	31800	2.7	
	C	240	21200	1.2	63
	D	678	38300	1.9	

A = Cysteine + X-irradiation (880 r)

B = Cysteine

C = X-Irradiation (880 r)

D = Control

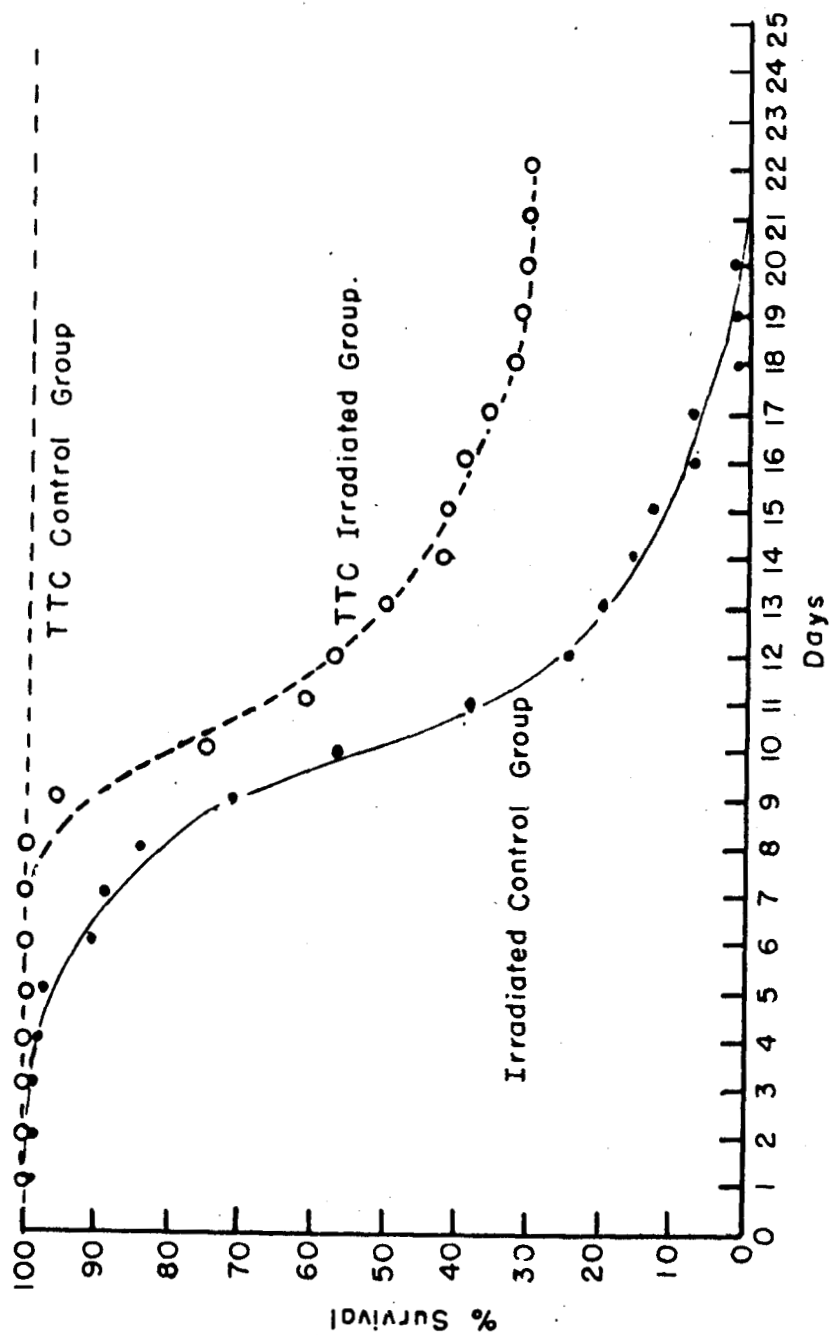


Fig. 1

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against Radiation," 11 July 1951. Onion epidermis cells were irradiated with 50 KV x-rays from an AEG 50-thin beryllium window tube. The irradiation effects were studied by the acridine orange staining technique. Epidermis treated with glutathione, cysteine, or acetone either before or immediately after irradiation showed high protection, while tissue treated with cystine, urea, or sodium chloride showed no protection. The protective effect was dependent upon the dose and time of application.

Abstract of AMRL Report 61, "Test Measurements on Scintillation Geiger Mueller Counters," 18 July 1951. Test measurements on commercially-available scintillation counters showed areas of high sensitivity. Some alteration in the counter's geometry and construction allows it to be used in special investigations such as the measurement of alpha activities in body cavities and tissues.

Quantitative Studies on the Effects of Non-ionizing Radiation on the Skin (was 6-64-12-08-2 and 6-64-12-08-4). Additional reflectance data on white and Negro skin (male and female) were obtained in order to establish the same statistical basis for the true skin colors and for the 2 sexes. The statistical evaluation of the sex factor is being determined. Experiments to give additional proof that the 3 reflection minima (near 980, 760, and 845 mu) are caused by water absorption bands are under way.

Abstract of AMRL Report 58, "A Quasi-null Method of Measuring Small Amounts of Radiant Energy," 11 July 1951. The Coblentz astatic galvanometer used in the method was found to drift and change sensitivity, probably due to variations in torque in the suspension. To alleviate this, a micro-screw clamp was incorporated which made it possible to adjust sensitivity without changing the relative positions of the sensitizing magnets. Since linearity was best near the center of the scale, a Wenner potentiometer was permanently added to the system so that readings could always be made near zero. The smallest division on the Wenner being equivalent to 3 cm on the galvanometer scale, and the fact that physical zero seldom coincided with scale zero, gave rise to the adjusted interpolation method of evaluating the energy.

Enzyme, Endocrine, and Metabolism in Total Body Irradiation (was 6-59-08-10). Protective Effects of Pharmacologic Agents. Aureomycin was studied for its possible protective effect against total-body x-irradiation in rats. Animals were given an x-ray dosage of 880 r. Aureomycin was administered alone and in combination with a prepared diet by stomach tube as well as in drinking water as 0.1 and 1.0% solutions. A slight prolongation of life was observed, but the survival rate (28 days) was the same as that of controls. In addition, the use of aureomycin drinking water in conjunction with pitressin injection 5 minutes before irradiation did not affect the survival rate usually found with pitressin pre-treatment. Intraperitoneal injections of aureomycin were attempted but were very unsatisfactory because the animals showed an intense reaction to the injection. Thyroid Response to Total Body X-irradiation. The changes in thyroid and serum I¹³¹ content of rats after total body x-irradiation at 1000 r indicate a stimulation of thyroid activity by 2 hours after irradiation (Table 2). This increased activity is apparent until 1 day after irradiation, from which time until the 6th day there is a progressive decrease in activity. These changes in functional activity of the thyroid are probably due to systemic damage caused by the radiation and are mediated through the hypophysis. The initial increased thyrotropin release from the hypophysis is followed by a shift of pituitary function toward increased adrenocorticotropin elaboration at the expense of thyrotropin production.

Abstract of AMRL Report 64, "The Protective Effect of Pitressin and of Epinephrine against Total Body X-irradiation," 18 September 1951. In experiments

TABLE 2

Time after Irradiation	Thyroid Gland I ¹³¹ Content % Change		Serum I ¹³¹ Content % Change
	Total	Organic	PBI
Zero	0	1	-4
2 hours	-6	10	32
1 day	0	-2	42
2 days	-21	-24	-67
3 days	-29	-27	-68
4 days	-68	-68	-73
6 days	-54	-46	-92

on total body x-irradiation, rats were subjected to a dosage of 880 r. Pitressin given 5 minutes before irradiation afforded considerable protection, with a survival rate of 28 days of 86% (12% for the control group). Similarly, pitressin injected 20 minutes before exposure decreased the mortality significantly. With injection 40 minutes prior to irradiation the protection was diminished but still definite. Pitressin given 5 minutes after irradiation conferred slight but definite protection. Epinephrine administered 5 minutes before irradiation also seemed to give protection but not to the same degree as pitressin. Threshold studies employing greater x-ray dosages indicated that injection of pitressin 5 minutes before irradiation raised the threshold from about 880 r to about 1100 r.

Effect of Irradiation on Single Cell Organisms (was 6-64-12-06-25). Yeast cells irradiated with sufficient doses of ultraviolet light showed a decrease in the SH-content as well as in the number of viable cells. Subsequent irradiation with visible light led to a partial reversal: an increase in the SH-content as well as in the number of viable cells. Yeast cells after ionizing irradiations showed a decrease in the SH-content and cell count. Subsequent irradiation with visible light had no effect on the SH-content of the cells but led to a partial increase in the viable count, depending apparently on traces of heavy metals present. Typical results are shown in Table 3. An amperometric method for the determination of sulfhydryl groups was developed, permitting the determination of sulfhydryl compounds in amounts of 15 gamma with an accuracy of 2%. Evidence has been obtained that this method will permit the determination of SH-compounds within the intact yeast cell.

Biophysical Study of Burns. A semi-quantitative orienting study of burns was undertaken on 30 animals (mice, guinea pigs, rabbits). Spectral reflection curves in the visible range (400 to 700 mu) were taken before and after burning. The animals were depilated in the region of the hip and thigh. Twenty-four hours later a pre-burn reflectance recording was made on the GE recording spectrophotometer. The post-burn reflection recordings were taken immediately after the heat exposure and periodically at 1-hour intervals thereafter for 4 hours, then at 24-hour intervals for up to 5 days. The burns were made under constant geometric conditions (time and temperature being the only variables) by means of hot air produced by the flame of a Bunsen burner and streaming through a metal pipe. The temperature range, measured at the place of exposure, was 150° to 260° C; the range of exposure time was 5 to 10 sec. The exposure at the lower temperature and the shorter time produced a light 2nd-degree burn; temperatures

TABLE 3

CHANGES IN THE CELL COUNT AND SULFHYDRYL CONTENT OF YEAST CELLS, AS A
 FUNCTION OF IONIZING AND ULTRAVIOLET IRRADIATIONS AND THE SUBSEQUENT EFFECT OF VITAMIN C

Initial Cell count	Initial SH-content %	Treat- ment (min.)	Surviving Cells	SH-content of surviving cells: %	Irradiation with visible light, min. ***	Surviving cells after irradia- tion with vi- sible light.
2.49 x 10 ⁹	0.21	X-5*	4.32 x 10 ⁸	0.12	30	6.4 x 10 ⁸
1.68 x 10 ⁸	0.26	UV-2**	5.5 x 10 ⁷	0.21	30	1.03 x 10 ⁸

* X-irradiated for 5 min: Machlett AEG-50 tube, thin beryllium window, operated at 15 kV, target specimen 10 cm. distant.

** Ultraviolet irradiation for 2 min: 15 W GE germicidal lamp 60 cm. target distance

*** Visible light irradiation. Light source: GE AH₄ lamp, with fixed reflector, target specimen 20 cm. through 1 cm. 5% CuSO₄ filter.

All specimens were irradiated in layers of 1 mm. depth.

above 200° C and exposures of more than 7 seconds resulted in charring or 3rd-degree burns. As shown by the reflectance recordings, the results could be duplicated fairly well. The pre-burn reflectance recordings on guinea pigs and rabbits showed a pattern similar to those on human buttocks which confirmed the measurements of Edwards and Duntley. On light burns an increase of reflectance was observed immediately after the exposure. It was followed by a decrease of reflectance throughout the entire range. On heavier burns, and when the skin was charred, the decrease of reflectance was observed immediately after the exposure; the heavier the burn, the greater the decrease. Although no detailed analysis of the reflectance curves has been made so far, the following observations on the post-burn reflectance recordings can be reported:

1. The absorption-caused dips at 542 and 576 mu are deepened.
2. The slope in the 600-700 mu range has become steeper.
3. Twenty-four hours and more after the heat exposure a dip in the 630-640 mu region appeared.
4. Three to 5 days after the heat exposure the curve in the 400-600 mu range becomes flattened, which seems to indicate that it represents the reflectance of the scar tissue.

Future. The DNA-cysteine-irradiation experiments will be concluded. The mouse-cornea studies will be continued. The mechanism of the protective action of TTC will be studied. The distribution of the formazan in different cell groups may prove to be of practical therapeutic value. The early effects of incorporated radioactive substances (polonium) will be studied with specially developed scintillation counters (see report).

The detailed study of the reflectance pattern in the 700 to 1000 mu range will be continued. Irradiation experiments with known amounts of radiant energy will be started.

Other pharmacologic agents will be tested for their protective effects against total body x-irradiation. The effects of total body x-irradiation on the interaction of various endocrine systems will continue to be studied.

Studies will be continued on the effects of radiation on yeast cells.

The instrumentation for producing heat erythema and/or burns under controlled conditions by the radiation of a heavy current carbon arc is nearing completion. A thermocouple to be used as the energy measuring device is being calibrated at the National Bureau of Standards. The influence of the unavoidable output fluctuations in the short exposure times to be expected will have to be examined; under consideration are measurements before and after exposures, and continuous recording.

Reports. AMRL Report 66, "Thyroid Response to Total Body X-irradiation," 28 September 1951 (see Progress, same title).

Other reports are listed under Progress and abstracts given.

Drs. Dripps, Greene, Hitchcock,
Karpovich, Navy, Orth

SECURITY CLASSIFICATION
PROGRESS REPORT (QUARTERLY)

RESEARCH AND DEVELOPMENT PROJECT CARD (NEW PROJECTS)		1. SEC.	2. PROJ. NO.
1. PROJECT TITLE Resuscitation		3. PROJ. NO. 6-59-09-1	
6. BASIC FIELD OR SUBJECT		7. SUB FIELD OR SUBJECT SUB GROUP	
8. COGNIZANT AGENCY	12. CONTRACTOR AND/OR LABORATORY		CONTRACT/W. O. NO.
9. DIRECTING AGENCY			
10. REQUESTING AGENCY	13. RELATED PROJECTS	17. EST. COMPL. DATES	
11. PARTICIPATION AND/OR COORDINATION	14. DATE APPROVED	RES.	
		DEV.	
		TEST	
15. PRIORITY	16.	18. FISCAL EST'S.	
19.			
20. REQUIREMENT AND/OR JUSTIFICATION			
21. BRIEF OF PROJECT AND OBJECTIVE			
<p>Dr. Dripps. Mechanical Respirators. The 1st problem has been to compare the effects of the such /sic/ and blow type of mechanical respirator with 1 offering intermittent positive pressure. Comparisons are being made of the Pneolator with the Emerson field model. The Stephenson resuscitator has been unsatisfactory from a mechanical standpoint. Anesthetized patients have been the initial subjects.</p> <p>Dr. Greene. A study of pulmonary edema, with special reference to the effect of mechanical artificial respiration. Since 1 July 1951 the following progress has been made: Study of best methods of producing pulmonary edema for the purpose of this investigation. 1) Inspiratory resistance was tried once unsuccessfully. The production of left heart failure by an overdose of nor-epinephrine was tried several times without success. These methods were discarded. 2) Alpha-naphthyl-thiourea (ANTU) was used; a fairly satisfactory type of pulmonary edema was produced by the intravenous administration of 50 mgm/kg. Other dosages were tried but were unsatisfactory. This dose is not uniformly successful in producing pulmonary edema; it has also produced sudden death in 2 dogs. 3) Mild pulmonary edema has been produced by a large intravenous infusion of physiologic saline. Further trials seem warranted.</p> <p>Methods of measuring pulmonary edema for the purposes of this investigation: 1) Auscultation of the chest has been used as a rough guide to the presence of pulmonary edema in dogs. Limitations are obvious. 2) Tissue sections have been made after those experiments in which the animal succumbed. These probably will not contribute materially to the final analysis but are useful controls of the work as it progresses. 3) Preliminary experiments with a method of measuring electrical conductivity through the lungs as a measure of their fluid content have been made. 4) The development of</p>			
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File: Research Program 30 Sept 1951

pulmonary edema has been followed in several dogs with measurements of blood pressures and blood gases in the pulmonary and systemic circuits. In other dogs the same observations have been made during the development of the edema, accompanied by the use of either the Burns valve or the E & J resuscitator. The results have not demonstrated a clear trend.

f. Future plans: Comparison of 2 or more respirators in the same animal with alternation from 1 to the other. The results would be followed with blood pressure and blood gas analyses as above./ Exploration of other methods of producing pulmonary edema./ Further tests of the electrical conductivity method of quantitating pulmonary edema.

Dr. Orth. Evaluation of Mechanical Respirators. Preliminary animal experiments have consisted in determining the possibility of dual artificial respiration efficacy with a single oxygen demand regulator and 2 pneophore valves. Separately used have been either of 2 type A-16 oxygen pressure demand regulators obtained from the Air Force. A metallic large-bore Y tube was placed beyond the regulator and from the 2 distal arms of the Y pneophore valves were placed. Each of the 2 valves then was connected to a dog into which an endotracheal tube had been inserted./ The dogs' respiration was greatly depressed or abolished with pentobarbital (35-50 mg/kg) given slowly intravenously./ Kymographic tracings from pneumographs, direct blood-pressure tracings, and blood gas analyses have been made.

Contrary to expectations, it has been found that a single demand regulator connected with an individual pneophore for each animal functions quite efficiently at pressures between 6 and 14 inches of water pressure per square inch. Such functioning occurs a) with both animals breathing spontaneously, b) with 1 animal breathing spontaneously and 1 having respiratory movements totally abolished, and c) with spontaneous respiration abolished for both animals. Individual characteristics of each pneophore valve are evident.

f. Future plans: To accumulate more specific data so that detailed analyses of pressure changes, blood gas alterations, etc., may be obtained. Particularly, simultaneous recordings of all the factors are needed. To extend the tests to more subjects using 3 and if possible, 4 dogs, simultaneously. Extension of the tests to humans should be possible in this period. Subjects in the State of Wisconsin General Hospital who have temporary respiratory cessation via barbiturates could be used, or neurosurgical patients in whom respiration has ceased due to the intracranial condition or intervention.

Dr. Delwin C. Stranda

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PROGRESS REPORT (QUARTERLY)

RESEARCH AND DEVELOPMENT / PROJECT CARD / (NEW PROJECTS)		2. SEC.	3. PROJ. NO.
1. PROJECT TITLE		4.	
Preservation of Blood for Transfusion		5. REPORT DATE	
6. BASIC FIELD OR SUBJECT		7. SUB FIELD OR SUBJECT SUB GROUP	
8. COGNIZANT AGENCY	12. CONTRACTOR AND/OR LABORATORY		CONTRACT/W. O. NO.
9. DIRECTING AGENCY			
10. REQUESTING AGENCY	13. RELATED PROJECTS		17. EST. COMPL. DATES
11. PARTICIPATION AND/OR COORDINATION	14. DATE APPROVED		RES.
	15. PRIORITY		DEV.
	16.		TEST
			OP. EVAL.
			Fy 18. FISCAL EST'S.
19.			
20. REQUIREMENT AND/OR JUSTIFICATION			
21. BRIEF OF PROJECT AND OBJECTIVE			
<p>Dr. Delwin C. Stranda, U. of Iowa. Anemia in patients with cancer, especially lymphatic, has been a study, begun under other auspices, will be the subject of a long series of experiments to be completed. Normal group O blood was irradiated in vitro with 1000 r x-rays and transfused to a normal recipient of group O. In a similar experiment performed with blood receiving 2000 r of x-rays in vitro, the blood was transfused. The donors' cells, as detected by inagglutinable erythrocyte counts, disappeared from the circulation of the recipients at normal rates, proving that x-rays had not damaged them appreciably.</p> <p>Four women with inoperable carcinoma of the cervix uteri, with no anemia initially and with no bleeding, served as experimental subjects. They, all of group O and were transfused with fresh blood from normal donors of group O. During the study the patients each received from 3000 to 5000 r of x-rays to the pelvis and 200 mg/hr of irradiation from radium implanted in the cervix. The rates of disappearance of the donors' cells were measured by analysis of the curves plotted from the inagglutinable erythrocyte counts. The concentration of the recipients' cells were calculated from total erythrocyte counts minus inagglutinable cell counts performed simultaneously. Equations were constructed to fit the loss of the transfused cells from the circulation. From these data calculations were made of the recipients' cell counts to be expected on the assumption that they were destroyed in a similar manner and that the rate of annual start of new erythrocytes proceeded at the same rate as previously. These expected recipients' cell counts were compared with the observed values. In 1 patient biologically tagged cells were destroyed in random fashion at an increased rate before irradiation. In 3 patients the transfused cells were lost at a normal rate until irradiation, when a random destructive process became operative. In all 4 patients the rate of erythropoiesis was accelerated, thus partially or completely compensating for the increased destruction of erythrocytes.</p>			
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Transfusion

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Conclusions: In 1 patient with carcinoma of the cervix uteri a hemolytic mechanism was present before irradiation which destroyed normal erythrocytes in a random fashion, in addition to their loss by normal aging. In 3 patients normal transfused cells survived normally until irradiation initiated a hemolytic mechanism which destroyed the cells at random, irrespective of their age. That the irradiation is not a direct effect on the transfused cells is proved by the normal survival of cells irradiated in vitro. The irradiation apparently releases a hemolytic factor from normal or carcinomatous tissue. Contrary to the opinion of many, erythropoiesis is actually accelerated and when anemia results, it is due to failure to compensate completely for the increased hemolysis.

Measurements of blood volume after transfusion. Two normal subjects of group A have been transfused with fresh group O blood collected in acid-citrate-dextrose mixture, U.S.P. The total blood volume has been measured simultaneously by the Ashby method, the radioisotope P^{32} , and by the Evans-blue-dye-and-hematocrit technique. Certain discrepancies which were apparent by comparison of the results of the 3 methods are being investigated further. These subjects will be followed with inagglutinable cell counts as part of the study on blood preservation. As soon as the techniques are perfected a large series of subjects will be transfused./ Work previously reported on the uptake of oxygen in human erythrocytes in Warburg manometers has been extended: Effect of heating erythrocytes. In 6 more experiments fresh washed human erythrocytes were heated by immersing their containers in boiling water for 10 minutes. The mean oxygen uptake, as read in 24 manometers, was 0.73038×10^{-3} microliters/ hour/ million cells, for the boiled cells and approximately the same for the controls./ Effect of freezing and thawing erythrocytes. Further experiments confirmed those reported; 3 more were performed in which fresh human erythrocytes were frozen and thawed in Warburg vessels. The control cells took up the usual amount of oxygen whereas those frozen and thawed showed practically no oxygen uptake./ Effect of atmosphere of oxygen. One experiment was performed in which fresh washed human erythrocytes were studied in an atmosphere of oxygen. They were found to have the same uptake of oxygen as those studied in air. Six experiments were carried out in which human red cells were heated for 10 minutes in boiling water and then studied in an atmosphere of oxygen. The mean oxygen uptake of the boiled cells was 1.49707×10^{-3} microliters/ hour/ million cells, as compared with 1.114925×10^{-3} for the controls in the same atmosphere. The uptake of the boiled cells in oxygen was about twice the uptake in air./ Effect of atmosphere of nitrogen. Seven experiments have been performed so far. In 18 manometers containing fresh washed human erythrocytes in nitrogen, as controls, there was an output of gas in the range of from 2.0721×10^{-3} to 4.1033×10^{-3} microliters/ hour/ million cells. This gas is thought to be oxygen. In 36 manometers in which were placed similar erythrocytes, which were heated in boiling water for 10 minutes, there was no perceptible change, either positive or negative, in the gas volume. This is in distinct contrast to the behavior of boiled cells in oxygen or air.

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AMSGS, Blades, Blake, **SECURITY CLASSIFICATION**
Breed, DeBakey, **PROGRESS REPORT (QUARTERLY)**

RESEARCH AND DEVELOPMENT PROJECT CARD (NEW PROJECTS)		2. SEC.	3. PROJ. NO. 6-59-12-22
1. PROJECT TITLE Traumatic Surgery and Shock		5. REPORT DATE 30 Sep 51	
6. BASIC FIELD OR SUBJECT		7. SUB FIELD OR SUBJECT SUB GROUP	
8. COGNIZANT AGENCY	12. CONTRACTOR AND/OR LABORATORY		CONTRACT/W. O. NO.
9. DIRECTING AGENCY			
10. REQUESTING AGENCY	13. RELATED PROJECTS		17. EST. COMPL. DATES
11. PARTICIPATION AND/OR COORDINATION			RES.
			DEV.
			TEST
		14. DATE APPROVED	OP EVAL
		15. PRIORITY	16.
19.			
20. REQUIREMENT AND/OR JUSTIFICATION Howard, Howes, Localio, Mayerson, McManus, MNL, MR&DBd, Selye, Smith, (Hugh), Soto-Hall, SRU			
21. BRIEF OF PROJECT AND OBJECTIVE AMSGS. Basic studies are in progress. Observations on the plasma volume and the concentration and total amount of circulating plasma protein before and after surgical repair of the fistula are being collected from arteriovenous fistula cases at Walter Reed Hospital. Laboratory studies are continuing on the alterations of volume and composition of the fluid compartments of dogs before and after severe hemorrhage. Equipment has been assembled for the simultaneous recording of blood pressure in several arteries and veins of dogs. f. Future. Increased attention will be given to the concentrations and movements of electrolytes in the body fluids. Experiments are being planned to study some aspects of vascular resistance in shocked animals. Observations on the actions of dextran and oxypolygelatin will be made. Use of the Artificial Kidney in Metabolic Problems. Purpose: 1) To treat patients with acute uremia and in so doing attempt: a) to develop a simplified and safe program of therapy; b) to identify substances which may be responsible for the clinical manifestations of uremia. 2) To treat patients with hepatic coma with and without oliguria and hypotension in order: a) to determine the value of the artificial kidney in such cases; b) to identify, if possible, the substances responsible for renal damage in liver failure or for other manifestations, e. g. fetor hepaticus./ 1) Study of acute uremia: a) Through experience with patients with acute uremia it is possible that management could be safely simplified to require fewer and less refined laboratory determinations. Use not only of the artificial kidney but of the intermittent peritoneal lavage method of Grollman is contemplated; b) The problem of identification of toxic substances in uremia includes: dialysis; anion fractionation of artificial kidney bath fluid, blood, and urine; and ultimate chromatographic and differential eluting techniques for actual separation and identification. 2) In investigating hepatic coma, aside from clinical experience with the artificial kidney, the anion fractionation			
22. JROB SN.	PC.	IC & P.	X. I. C.

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method is again contemplated with pre- and postdialysis comparisons of blood and urine. Electrolyte variations will be measured as well./ One patient in hepatic coma was studied since previous report. The patient expired before hemodialysis could be undertaken./ A detailed plan for metabolic study of a patient with hepatic coma has been completed. Liver Function during and following Anesthesia and Surgery. Purpose: To determine the effects of various types of anesthesia, duration of anesthesia, and surgical procedures upon liver function. Patients are selected from the operation schedule at Walter Reed Hospital. Blood samples are taken pre-operatively, after induction of anesthesia, and at intervals following surgery and a panel of liver function tests are performed. This includes serum bilirubin, cephalin cholesterol flocculation, thymol turbidity, Kunkel gamma globulin, and the serum cholinesterase. Bromsulfalein (BSP) tests are determined pre-operatively and at intervals postoperatively in selected patients. Estimations are also made of the blood keto acids by means of paper chromatography and plasma prothrombin by a 2-stage method in a limited number of surgical patients. The selection of patients now includes those with major thoracic and abdominal surgery instead of the minor procedures previously studied.

Dr. Blades. Summary of article for publication, "Experimental Studies with Intra-Arterial Transfusion Overtransfusion of the Normal Dog," by Beattie, Thistlethwaite, Blades, and Wood: Overtransfusions of whole blood were given into the femoral artery of normal dogs. The percentage increase in blood volume and the speed of transfusion were found to be major factors in their survival. When an increase in blood volume of approximately 100% was given in 30 to 60 minutes, the mortality rate was 30%. The animals survived an overtransfusion of 25% to 35% of their blood volume given in 4 to 5 minutes. The effect of overtransfusion upon respiratory rate, venous pressure, pulse rate, and blood pressure were described. The venous pressure reflected an overtransfusion. In these experiments the venous pressure returned to normal levels 10 to 15 minutes after the transfusions were completed. Animals died from acute cardiac failure or from signs of plethora and cerebral damage./ It is proposed to make a group of 10 dogs hypoproteinemic with total proteins under 5 grams per cent. The technique developed here is to draw whole blood and to reinfuse the red blood cells suspended in a saline solution. The dogs entering this colony have hematocrits at admission averaging 32 to 37, and their total protein ranges from 7 to 7.4 grams per cent. They have all had normal albumin and globulin ratio./ After 2 weeks in this colony 5 dogs who have had the plasma removed from 200 cc of their blood have shown resultant hematocrits varying from 37 to 46. Their total proteins have dropped to 6.0 to 7.2 grams per cent. In all dogs the A/G ratio has reversed. After a 2d bleeding their hematocrits have risen even higher to 41 to 59 and their total proteins have fallen. It is planned to continue the bleeding until the total proteins are under 5 grams per cent. The animals will then be given compatible blood transfusions until their blood volumes have been doubled in approximately 60 minutes.

Dr. Blake's contract was initiated 1 May 1951 to study the effects of different methods of increasing plasma volume on renal hemodynamics and electrolyte excretion./ No satisfactory assistant was available until 1 September. Training in the techniques involved requires about 6 weeks.

"Renal Function in Surgery" by Dr. Breed et al., to be published in Surgical Clinics of North America, ends with this discussion: What has renal function data and more particularly, clearance data, contributed to the management of the surgical patient?/ Aberrations in kidney function normally occurring in different age groups emphasize the fluid replacement hazards in pediatric and geriatric patients. In the pre-operative preparation of the normal patient, dehydration, starvation, and the common pre-anesthetic medications produce a decrease in renal function. Renal clearance data elucidate these changes. Even though depression in function may be moderate, the surgeon is challenged to prepare his patient in such a way that this important defense to stress offers the widest margin of safety.

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The patient who has a vague history of vomiting or who has been draining indeterminate amounts of fluid from a fistula, on clinical examination, may show deceptively little, but the impending severity is demonstrated by clearance data approaching shock-like values. Without proper electrolyte therapy the simple operation of closing the fistula may result in severe shock and anuria. Under certain conditions, cyclopropane, thiopental, and ether anesthesia may produce the renal pattern of marked reduction in clearance with rebound to normal after the agent has been discontinued. The fact that values return to normal so quickly after anesthesia indicates there has been no renal damage. However, in view of this depression of clearances due to anesthesia, one wonders whether added insult during operation, such as incompatible transfusion, would be more likely to produce anuria than under circumstances where the renal function is normal. It might be well to emphasize the reasons why, in the past few years, there has been such a marked reduction in the mortality rate of anuric patients. In 1946, Lucke reported a mortality rate of over 90%. In 1950, Bull et al. reported a survival rate of over 90%. This improvement has been a result of 3 therapeutic factors: 1) a protein-sparing, electrolyte-free diet, high in carbohydrate and fat which prevents the lethal rise in the level of blood K; 2) the prevention of pulmonary edema by limiting fluid intake to about 750 cc per day; 3) adequate fluid electrolyte and diet replacement, after the onset of a good diuresis. The clinical course of the patient's illness has been correlated with a characteristic pattern. This correlation emphasizes that careful clinical appraisal of the patient, plus such simple tests as blood pressure, accurate urine volume, specific gravity and routine blood chemistries, are sufficient to evaluate and treat intelligently common fluid balance problems in surgery.

Report received 17 September on Physiologic Studies of Traumatic and Operative Shock. Since May the effect of electrolyte and water replacement on renal function in several cases of shock has been studied. This approach was stimulated by a case of moderate shock characterized by sodium loss from gastro-intestinal drainage associated with low blood pressure. Features of this case are presented:

Type of data	Control observations	Therapy:	Observations:	
		280 cc sodium lactate at 5.95 cc/min	Minutes after completion of lactate	
			18 minutes	78 minutes
Extraction ratio of PAH in %	85.75; 81.4		77	-
Clearance of PAH in cc/min	213; 220		617	366
Plasma sodium in mEq/l	118			133

The almost normal extraction ratio at the time the patient showed cold extremities, mental lethargy, and a weak pulse together with a 60% reduction below normal in the renal plasma flow, stands out in contrast to the reduced extraction ratio observed at the time the patient's extremities were warm, the pulse bounding, the mental status considerably more alert, and the renal plasma flow normal.

However, it is difficult to say whether this drop was due to a spontaneous downward trend or to the fairly rapid administration of a concentrated sodium ion, so that AV shunts (not of the Trueta type) as described by Cargill and Mitchie when they gave albumin rapidly (sic). At any rate the renal plasma flow was increased for several periods but dropped again in spite of the return to more nearly normal sodium levels. The woman improved rapidly and left the hospital but repeat studies are planned to determine her postshock status

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A similar study on a healthy elderly male being examined for other purposes yielded these data:

Type of observation	Control	200 cc of molar Na lactate at 7.7 cc/min	22 minutes after completion of Na lactate
EP PAH	93.6		95.7
C PAH	632		775

Here, in a nonshock individual with a normal renal plasma flow to begin with, the increased renal plasma flow in response to lactate did not change the extraction ratio of PAH. Since isotonic saline, when given rapidly, increases the filtration rate in dogs and man, it was questioned whether or not it would cause a rise in renal plasma flow and a drop in extraction ratio of PAH in normal man. Consequently information was obtained on a young male convalescing from an appendical abscess:

Type of observation	Control	After infusion of 1000 cc of isotonic NaCl in 25 min. at 64 cc/min	After total of 2112 cc in 33 min. at 63 cc/min	6.5 min after total of 3 liters NaCl completed	22 min after 3 liters completed
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EP PAH	90.3	87.8	84.3	90.3	90
C PAH	484	517		622	559

Apparently the extraction ratio can be pushed around in the kidney of normal people by powerful hemodynamic stimulants such as massive saline infusions or albumin and will return to its original condition in a very brief time. The usual detailed study of urea, electrolytes, inulin, and creatinine were done on all these patients. Quite a few interesting cases will be summarized in the next report. The paper on the effects of cyclopropane, ether, and thiopental on renal function by Habib, Popper, Bradley et al. appeared in Surgery in July. Our extraction ratios under anesthesia have been used as the basis for establishing the validity of the markedly reduced renal plasma flow which they found consistently. Both the original and some more recent work of our own suggests that such consistency is not usual and that there might be considerable variations in clearances, perhaps due to such circumstances as varying degrees of pre-operative hydration of the patients.

g. Reports. Data on extraction ratios under anesthesia are almost sufficient to warrant a note for Proc. Soc. Exp. Biol. & Med. A requested article for Surg. Clinics of No. America (November) entitled "Renal Function in Surgery," will review all the clearance data on patients with surgical conditions. This information will be correlated, if possible, with the usual clinical laboratory data with the aim in mind of a better understanding of the pathologic and physiologic factors in relation to the indicated therapy.

"The Combined Effect of Cortisone and Partial Protein Depletion in Wound Healing," Findlay and Hoxes. Summary. In rabbits it was necessary to cause more than 20% weight loss by starvation and protein depletion to retard the initial appearance and growth of granulations in the healing wound. The healing of wounds in animals partially depleted of protein and losing less than 20% by weight was retarded by low doses of cortisone, provided a low-protein diet was given after wounding. Debility and inadequate intake of protein augmented the action of cortisone on wound healing. On the contrary, healing was not retarded, provided a normal diet was consumed after operation. This may partly explain conflicting reports on wound healing associated with cortisone and ACTH administration. The mortality was greater in wounded animals receiving cortisone after being depleted on a low-protein and starvation regimen than when cortisone was not given. Conversely, cortisone did not help the wounded animal to sustain stress under these circumstances. The criterion used for the gross appearance of granulations may be defective. Histologically, extravasation of whole blood may masquerade, under certain circumstances, as granulations. The relationship of total circulating

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serum albumin to the healing of the wound and the diet was demonstrated.

Progress reported in this project on Wound Healing from 1 January to 31 August: Vitamin B₁₂. Since numerous investigators have found vitamin B₁₂ to be a growth factor and some have presented evidence of its importance in protein synthesis, its effect on wound healing was investigated in rats fed a normal and a low-protein diet. Healing rate was measured by testing the tensile strength of incised abdominal wounds on the 6th postoperative day./ The vitamin had no effect in the protein-depleted animals but it increased the healing strength of the wounds in those fed control diets. The amount of increase was statistically significant./ To corroborate this, vitamin B₁₂ was given to rabbits with ear wounds and the character of healing was observed grossly and through the dissecting microscope. Attention was directed toward the vascular response, the collection of extravascular material, and the growth of the blood vessels. Photographs were taken. These findings are being checked histologically and the extent of fibroplasia is being evaluated. Evidence has been obtained that B₁₂ favors an early increase of extravascular material and vascular growth./ If these results can be consistently reproduced in the future, then this vitamin seems to be a substance that shortens the latent period of healing.

Cortisone. It was demonstrated that the delay in the appearance of granulations is prolonged in proportion to the amount of cortisone given parenterally. Yet granulations finally appear even with extra-large doses, suggesting that its action was neutralized in some way. But even a small amount of cortisone applied locally inhibited granulations as long as it was applied. This action suggests that cortisone acts directly on the tissues and further that the neutralization of parenterally administered cortisone must be a chemical change of 11-dehydro-17-hydrocorticosterone./ Consequently, substances were sought that might neutralize cortisone given parenterally. Pituitary growth hormone, desoxycorticosterone, vitamin C, and many other substances were tried, but they failed to offset the capacity of cortisone to delay the appearance of granulations./ More accurate knowledge had to be obtained about the minimal amount of cortisone that would delay the appearance of granulations and its pertaining circumstances (sic), i.e. the relationship of the minimal dose to the nutritional state of the animal, the character of the diet, and the relationship of infection elsewhere. To establish criteria for future studies on healing in rabbits fed a low-protein diet, the degree of hypoproteinemia and the blood volume changes were determined and correlated with weight loss./ Total circulating proteins, especially the albumin, became depressed early and remained so unless a normal diet was again given. The changes in the blood volumes and the red cell mass did not follow a consistent pattern. Depression of the serum albumin did not adversely affect healing as determined by tensile strength unless the rabbits also lost at least 20% by weight./ Having this information, the effect of the combination of cortisone and hypoproteinemia was studied on open ear wounds in rabbits that lost less than 20% in weight. These observations were made: Rabbits fed a Rockland Chow diet throughout the test showed good granulations in their wounds by the 4th day./ Those fed a low-protein diet postoperatively until they lost from 15 - 20% in weight did not show granulations until the 5th or 6th day./ Rabbits fed a normal diet but given cortisone, 1.5 mg/K daily after operation, started to granulate their wounds on the 5th or 6th day./ Rabbits maintained on a low-protein diet before operation until they lost 9 - 14% of their weight and given 1.5 mg of cortisone/K daily and the same diet after operation did not show granulations on an average for 10.2 days. The granulations never became thick, usually remained pale and dry. Epithelization, however, progressed at a normal rate. The mortality in this group was considerable./ Rabbits fed the low-protein diet preoperatively until weight loss ranged from 10 - 16% and given the same cortisone dosage but Rockland Chow Diet postoperatively showed granulations on the 5th day./ These experiments show that a previously ineffectual dose of cortisone (1.5 mg/K) delayed

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The appearance of granulations if the animal was sustaining weight loss or was in negative nitrogen balance. Either extreme weight loss or extreme negative nitrogen balance alone delayed the appearance of granulations, but a moderate degree of either did not, unless coupled with a very small amount of cortisone. Furthermore, this delay could be offset by a normal diet after operation. The mechanism of action of cortisone was studied from the anatomical viewpoint:

With Cortisone
exposed blood vessels
no extra-arterial substance

Plaques of fibrin present
Interarterial area glazed
Many new blood vessels visible
under dissecting microscope

Without Cortisone
No exposed blood vessels
Extra-arterial substance has
filled space between vessels
Plaques of fibrin present
Interarterial substance becomes opaque
Many new blood vessels can be seen
sprouting from old vessels under dissecting microscope.

Considerable work in the past 6 months has been devoted to developing new methods. Chronic inflammatory change elsewhere in the body is known to delay healing, as does cortisone or weight loss. Whether a resulting hypoproteinemia or weight loss or a disproportion between body weight and cortisone is the cause of this delay is not known. Since variable degrees of bacterial infection are often obtained experimentally, the technique was used here of producing a concomitant turpentine abscess in the thigh of the rabbit. Although results are not fully evaluated, there is an early deposition of an opaque extravascular substance in the wound and not a delay in the appearance of granulations. All the vessels become dilated and tortuous during the 1st 4 days of healing and many more are visible, but then they tend to disappear again even though the thigh abscess is increasing in size and the animal has fever. Work is continuing on combinations with cortisone, vitamin B12, weight loss, and hypoproteinemia to determine possible synergism and antagonism. In reports on the prevention of peritoneal adhesions by cortisone, either irritants such as talc or a localized intraperitoneal infection that does not always produce adhesions have been used. To test these reports, a limited area of the parietal peritoneum of the rat has been burned with 10% silver nitrate; this invariably produced adhesions. But it has been impossible to prevent the formation of adhesions with adequate amounts of cortisone used locally or given systemically. Since rat is particularly resistant to cortisone the work is being transferred to rabbits. In the days immediately following the burn a considerable amount of fluid accumulates in the peritoneal cavity. Since this fluid can be collected and analyzed for electrolytes and protein, this method should permit the quantitative study of the loss of these substances from the host with either a chemical or a thermal burn. Lastly, the method can be used in combination with the caround technique to evaluate the influence of metabolic changes on healing. Cortisone by mouth, cortisone F, and ACTH in depository doses have been shown to delay the initial appearance of granulations. Amounts required have been studied; about 3 times as much was required by mouth. Reappearance of granulations after the cessation of administration of Adactar (ACTH depository) does not occur as rapidly as after cessation of administration of cortisone.

Although preliminary studies on nitrogen metabolism, using N15 Glycine, have been done on humans, it is impossible to complete them because work in the metabolism unit of Presbyterian Hospital has been suspended temporarily. Interest was directed toward determining intracellular water volume, by evaluating during the 1st 10 days after wounding the quantitative changes in this compartment based on the intracellular chloride space. Although tissue and serum sodium and potassium values were as expected, the chloride determinations were unusually abnormal. As a result, calculations of intracellular water were out of line. Therefore it was concluded that the use of the chlorides to determine intracellular water was not valid. A

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similar conclusion has been reached by those using the thiocyanate space. The only other reagent that would be applicable would be inulin, but this would entail too much time and money to justify results. This phase was terminated.

8-Azaguanine (Triazolopyrimidine, Guanazolo) It was demonstrated that 7.5 mg/K per day of 8-azaguanine given twice daily intraperitoneally was effective in delaying epithelization whereas this amount was not effective in a single dose. Believing that a satisfactory blood level of 8-azaguanine is required to inhibit epithelization, this same dose was given in oil intramuscularly. It inhibited epithelization. The minimal and maximum dose required in oil as well as the frequency of administration have been investigated and resulting changes instituted in the muscle at the site of injection also studied. An abundance of nucleic acid administered by mouth or by injection was shown to offset the action of 8-azaguanine on epithelium. Adenine, not guanine, administered by injection offset its action also. This result, obtained in mammals, is the opposite of that obtained by Kidder for tetrahyma. On the other hand, the work of Brown et al. with labeled guanine, also done in mammals, substantiates our findings. Neither adenine nor guanine alone inhibited epithelization. 8-aza-adenine did not inhibit epithelization. Neither 8-aza-adenine nor 8-azaguanine inhibited the initial appearance of granulations. 8-azaguanine given by mouth inhibited epithelization. Approximately 3 times as much had to be given. The durations of inhibitions of epithelization that can be obtained with 8-azaguanine is being studied. Weight loss with 8-azaguanine was most marked with single large doses given intraperitoneally. Weight loss was less with intramuscular injections in oil and multiple injections. Weight loss also occurred when 8-azaguanine was given by mouth. 8-Azaguanine and Cortisone. Both chemicals combined in proper amounts inhibited both epithelization and fibroplasia. In higher doses all animals died.

Dr. Localio. Alarm Reaction and Wound Healing. The technique used here for making standard wounds and testing their tensile strength has been perfected. The control curve of tensile strength in normal rats has been completed for postoperative days 3, 5, 7, and 10. In addition the 1st hormone, desoxycorticosterone, has been administered to a series of 32 rats; tests on the effect of open wound healing are nearing completion for the same number of postoperative days as above. Considerable experience has been acquired with the application of eosinophil counts in rats. To date it appears that this test is too sensitive to minor degrees of alarm for our purposes. However, studies will be continued.

Dr. Mayerson. Blood Volume Studies. Exchange of Albumin between Plasma, Interstitial Fluid, and Lymph. The results of the intravenous injection of serum albumin iodinated with I^{131} have been published in Amer. J. Phys.: 165, 15-26 (1951). For similar studies, in which the iodinated albumin was injected intraperitoneally and subcutaneously, data are being analyzed. Exchange of Globulin between Plasma, Interstitial Fluid, and Lymph. The Abbott Laboratories preparation of iodinated gamma globulin has been used in a manner similar to our preparation of iodinated serum albumin and experiments indicate that it leaves the blood stream at the same rate as the albumin. Its uptake by the lymph is also similar. This unexpected similarity in the handling of the 2 proteins suggests the desirability of testing other preparations. A suitable supply of canine globulin will be iodinated and used in the same manner. Varying amounts of concentrated serum albumin have been infused into anesthetized dogs and the consequent volume shifts studied. Lymph proteins and flows have been followed. It may be possible to quantitate the volume of interstitial fluid available for exchange with plasma. This fluid has been designated "capillary filtrate" to distinguish it from lymph, which is interstitial fluid that does not exchange directly with plasma. Further experiments are planned. Work on Infusion of Saline Solutions. A manuscript covering this virtually completed phase has been prepared for publication. Summary: Dogs, anesthetized with pentothal, were infused with volumes of physiological saline solution amounting

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to 1 to 3 times their plasma volumes at rates varying from 10 to 50 ml per minute. Judged by changes in the rates of disappearance of intravenously injected radioactive iodo-albumin, these infusions increase the transudation of albumin from the circulation as much as 3 to 5 times. Concomitantly there is an increased rate of albumin return via lymph approximately 3 times that of the pre-infusion period. This increased albumin return masks the true loss of albumin from the plasma, and in many cases, actually exceeds the loss and results in increased (20%) levels of total circulating albumin. If the lymph is not returned to the circulation or if the lymph is replaced by saline, the total circulating plasma protein is decreased by 15 to 30% in 3 hours. Specific activity data indicate that no new albumin is involved in the process but that the infusion serves to mobilize interstitial protein for return via lymph to the blood stream./ It is planned to continue with iodinated albumin, globulin, and protein with particular reference to various types of circulatory failure and to burns. Previous work has given approaches which may yield fruitful information of a quantitative as well as a qualitative nature as to fluid and protein shifts, capillary permeability, and importance of lymph transport in these conditions.

This report to 31 August on Dextran is based on 10 experiments in which 6% C.S.C. clinical dextran (Lot #84/21A) was injected intravenously into dogs under pentothal anesthesia in doses of 10 ml per K.B.W. The thoracic lymph duct was catheterized in 7 of the tests and, in 5, urine was also collected by catheterization of the ureters. All lymph not needed for analyses was re-infused. The duration of 8 experiments was between 5 and 6 hours; 2 were run for 24 hours after the infusion was given. Plasma volumes were determined by the use of T-1824 and hematocrits by the Win-trobe method. Dextran was determined by the method of Bloom and Wilcox. Howe's method was used for proteins. Blood samples were obtained by an indwelling needle in 1 of the femoral arteries. Blood pressure was measured with a mercury manometer from 1 of the carotid arteries./ The plasma volume was usually measured just before the start of the infusion, 30 minutes after its finish, and 2 to 3 hours after./ In every experiment there was an increase in plasma volume which was equal to or larger than the volume infused as measured 30 minutes after the end of the infusion. Two to 3 hours later, the plasma volume had always diminished to the pre-infusion level. The rate of lymph flow was usually diminished during or immediately after the infusion and then increased. This may indicate an initial shifting of fluid into the circulation because of initial high osmotic pressure. More data are needed to establish this relationship./ Blood pressure. The infusion usually raises the carotid blood pressure from 5 to 15 mm. Hg. The raised level usually persists as long as the blood volume is high and usually is back to the pre-infusion level in several hours./ Plasma. The plasma disappearance curves and other data are shown in figures omitted here..The 1st and steeper slope lasts for from 50 to 160 minutes (average 105) after beginning of the infusion and gives way to a smaller slope which continues for at least 24 hours. The data are consistent with the belief that the first slope represents the rapid transudation of the smaller molecules during and immediately after the infusion. The 2nd slope probably indicates the rate of metabolism of the larger molecules as the latter are broken down to smaller units and leave the circulation./ Lymph. The time after infusion at which perceptible amounts of dextran appear in thoracic duct lymph is variable. There is no question, however, but that it begins to leave the circulation immediately, since in at least half the experiments, measurable quantities appeared before the infusions were finished. Thereafter the concentration of dextran in lymph increases gradually to a peak at somewhere between 25 and 85 minutes. This level is then maintained for several hours; then it falls gradually. The change in slope of the lymph curves frequently coincides with the change in the plasma disappearance curves, again suggesting that there is a rapid transudation of the smaller molecules from the blood stream during the 1st hour or so and that the dextran then achieves an equilibrium between plasma and lymph.

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After an initial lag, there is established an equilibrium between plasma and thoracic lymph. With the dosage used, the concentration of dextran in lymph never approached that of plasma at any point in the experiment. In view of the relatively small amount of lymph appearing per unit of time, the actual amounts of dextran involved in the exchange between plasma and lymph are small./ Urine. The patterns of excretion of the dextran are variable, but in every experiment there was a marked excretion during the 1st hour after the infusion, amounting to between 5 to 8% of the dextran injected. In 1 experiment this level was maintained during the 2nd hour after. In the remainder, the excretion diminished gradually so that at the end of 6 hours after, less than 2% of the dose was found in the urine. In experiment 9, during 251 minutes 33.9% of the injected dose was excreted. At the end of 24 hours, the concentration of dextran in urine is quite low. The excretion during the last hour of this period represents less than 0.1% of the original dose. As to the relationship of dextran flow in the urine and in the lymph, while the patterns are similar, the amounts involved are much greater in urine than in lymph. Even at 24 hours, the concentration of dextran in urine is about 4 times that in lymph./ Distribution of dextran. Although inherent errors in the methods used preclude obtaining rigid quantitative data, the analysis indicates that much, if not most, of the dextran can be accounted for in the plasma, urine, and lymph and that there can be no large amount remaining in tissues. Previous work indicated that thoracic duct lymph may represent approximately 1/3 of the total volume of lymph circulating in the body. If this additional volume of dextran is added, it would seem that between 90 to 95% of the injected material can be accounted for in the 3 tissues studied. While the excretion of dextran in bile has not been studied in detail, several checks at the end of experiments indicate the presence of carbohydrate (presumably dextran) even as late as 24 hours after the infusion. This phase is being studied in more detail. Similar experiments with C^{14} labeled dextran should help to substantiate much of these data as well as to indicate the distribution of dextran in other tissues./ The experiments are being continued with variation in the amounts of dextran infused as well as using animals in hemorrhagic and traumatic shock.

McManus. Histochemical study of Inflammation and Repair. The ear wounds in 22 rabbits are available for study. These range in time from 1 to 30 days after removal of a segment of skin. On 12 of these animals enzyme material has been collected from the site of the wounding. The principal enzyme studies covered 3 of the alkaline phosphatases: phosphomonoesterase I, hexosediphosphatase, and 5-nucleotidase. These phosphatases show different distributions in the normal tissue and in the healing wounds./ Twenty-two tongue wounds in rabbits ranging in time from 1 to 11 days after incision have been studied. These have been followed by the alkaline phosphatase method, etc./ Twelve hypophysectomized rats and 12 controls of the same strain were given back wounds. The hypophysectomized rats show a considerable retardation of healing, associated with failure of appearance of concentration of enzyme equivalent to that found in normals. Formation of granulation tissue does not appear to be curtailed./ Subsequent studies will utilize the freezing-drying apparatus of the Altman-Gersh pattern.

The studies have shown to date a very marked difference in the healing of an ear wound and a tongue wound in the same animal. The basis for this is being explored. One of the 1st possibilities is the deficient vascularity of the rabbit ear as compared to rabbit tongue. The ear wound is made by the removal of a rectangular portion of skin which leaves a base resting on the perichondrium. The tongue wound on the other hand has no tissue removed. It is a simple incision and healing appears to proceed by 1st intention. To date the formation of fibrous tissue in the tongue wound is not associated with the presence of alkaline phosphatase while in the healing of the rabbit ear it does appear so associated./ Progress for 1st quarter: collection of material and investigation of methods; the development of 5-nucleotidase as a histochemical method; adaptation of lead tetroacetate as an oxidizing agent.

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MNL (conducted at Medical College of Virginia, Dr. Evans collaborating) Analysis of the kinetics of P^{32} metabolism in man was extended. The excretion of P^{32} has been analyzed along the lines of Sprinson and Rittenberg. From these analyses have been calculated the rate at which P^{32} leaves the body (0.8% per min.), the size of the labile body phosphorus pool (1.3 gm), and the rate of phosphorus turnover in the normal adult male (0.7% per min.). The body phosphorus pool is turned over about 10 times per day. Partition of P^{32} among the various fractions of plasma and red blood cells have been investigated. In plasma, it was found that the inorganic fraction accounted for some 95% of all the P^{32} found at any time after injection while the trichloroacetic acid insoluble organic fraction accounted for almost all the rest. Only a minute amount of organic phosphorus was found in the so-called acid soluble organic fraction, while the P^{32} level in this fraction was so low it was undeterminable. The question is whether this classically accepted phosphorus component actually exists in plasma or is merely an experimental artefact. Red cell data have shown that approximately 85% of the injected P^{32} is found in the inorganic form at peak uptake while most of the remainder is found in the acid soluble organic fraction. This would seem to establish definitely the presence of the inorganic component, the existence of which in the red cell has often been questioned. Neither insulin nor glucose exert any experimentally unequivocal effect on the P^{32} partition in either plasma or red cells.

Residual Nitrogen of Plasma of Rats and Human Subjects following Burns. This study, previously done in rats, is being extended to humans. The ultrafiltrates of human plasmas are being analyzed for urea, uric acid, creatine, creatinine, alpha-amino nitrogen, and amino nitrogen after hydrolysis of the ultrafiltrate. The aim was to determine whether a polypeptidemia occurs in human plasma after severe burns, comparable to that found in rats, and whether this polypeptide component was identifiable as that fraction known as "residual nitrogen." Thus far too few burn cases of sufficient severity have been available to allow definite conclusions.

f. Future. The basis for the changes in absorption spectra of plasma ultrafiltrates seen after burns and their relation to "residual nitrogen," is under investigation. These techniques will also be utilized: Fractionation and quantitative analysis of individual amino acids of both burned and normal ultrafiltrates before and after hydrolysis, by microbiologic and chromatographic techniques. This will yield information as to the amino acid differences in burned and normal ultrafiltrates and the composition of the abnormal polypeptide fraction. The possible relationships of this component to the patient's clinical course will be determined. Measurements of uric acid by the spectrophotometric method developed in this laboratory will be continued in patients with various types of injury as a part of a broad study of metabolic changes following trauma. Clinical trial of the apparatus developed for the rapid transfusion of whole blood will be initiated. The relationship of irreversible shock to the liberation or activation of proteolytic enzymes, and the possibility of developing anti-enzymes will be investigated.

g. Reports. "A Spectrophotometric Method for the Determination of Plasma Filterable Uric Acid," Rosen, H. and Levenson, S. M., MNL Rept. No. 86, 30 August 1951

"The Nature of the Undetermined Nitrogen in Serum of Rats following Burns," Ibid., MNL Rept. No. 87, 10 September 1951.

Summary of 1st report: Uric acid has a high extinction coefficient in the ultraviolet. Advantage of this was taken in devising a method for determining plasma filterable uric acid. It was observed that determinations of the absorption by plasma ultrafiltrates at 293 m and 305 m constitute a direct measure of uric acid. Recommendations: The spectrophotometric method for determination of plasma filterable uric acid presented in this report is simple and reliable and should be included in the Army Laboratory Manual.

Summary of 2nd report: "Undetermined" nitrogen constitutes a large part of the azotemia associated with severe burns, and there is a close correlation between this fraction and mortality. Ultrafiltrates of serum from normal and burned rats

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were utilized to determine the nature of this component. These observations were made: Ultraviolet absorption spectra of the ultrafiltrate before and after irradiation with ultraviolet light; analyses of total filterable nitrogen, urea nitrogen, and - amino nitrogen before and after acid hydrolysis./ It is clear from these data that the "undetermined" nitrogen occurring in the serum of burned rats is combined amino nitrogen, probably peptide in nature. The fraction occurs naturally in rat blood to the extent of 20% of the nitrogen filterable through Visking sausage casing under pressure. This percentage is not altered in blood of rats subjected to severe burns, but rises proportionally with the total filterable nitrogen. Recommendations: Observations of this type should be extended to patients with thermal and radiation injury as part of an investigation of nitrogen metabolism after trauma.

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Dr. Bean, AFIP,
U of Iowa
Bang, Capps,

SECURITY CLASSIFICATION
PROGRESS REPORT (QUARTERLY)

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RESEARCH AND DEVELOPMENT PROJECT/CARD/NEW PROJECTS		1. SEC.	2. PROJ. NO. 6-60-09-11
1. PROJECT TITLE Acute and Chronic Diseases of the Liver		5. REPORT DATE 30 Sep 51	
6. BASIC FIELD OR SUBJECT		7. SUB FIELD OR SUBJECT SUB GROUP	
8. COGNIZANT AGENCY	12. CONTRACTOR AND/OR LABORATORY		CONTRACT/V. O. NO.
9. DIRECTING AGENCY			
10. REQUESTING AGENCY	13. RELATED PROJECTS		17. EST. COMPL. DATES
11. PARTICIPATION AND/OR COORDINATION			RES.
			DEV.
			TEST
		14. DATE APPROVED	OR EVAL
		15. PRIORITY	16. PISCAL EST' S.
19.			
20. REQUIREMENT AND/OR JUSTIFICATION Davidson, Gyorgy, Havens, Klatskin, Neefe, Oliphant, Post, Reinhold, Stokes, Turner, Gordon, Walter Reed Army Medical Center, Watson.			
21. BRIEF OF PROJECT AND OBJECTIVE Dr. Bean. Hormonal, Metabolic & Nutritional Factors. In progress when this project received a new contract number: 1. Preliminary experiments on administration of an antipantothenic acid compound, pantoyletaurine, in man, with emphasis on acetylation. 2. Study of the collateral circulation in cirrhosis of the liver, especially with reference to abdominal vessels. 3. Continued studies on intravascular pressure in the lower bowel. 4. Detailed survey of the incidence of palmar erythema, senile angioma, and caviar-like lesions under the tongue. 5. Continued studies on methods for determining various hormones. 6. Studies on clubbing and hypertrophic osteoarthropathy. This work was reported on 1 October: Publication of 2 articles on electrophoresis, comparing serum and plasma values, a study of absorption from the gut using portal anastomotic veins. Reports on 5 years' study of 17-ketosteroids, on vascular spiders in children with hepatitis, on adenylic acid and cozymase, and on metabolic data in 3 patients with Wilson's disease are being written. Our major activities now deal with steroid hormones in chronic liver disease and use of vitamin-B antagonists in human subjects./ Corticoid hormone excretion in liver disease is being studied by the Talbot method. Elevated or normal values are found for cirrhotics, in contrast to the depressed 17-ketosteroid output observed in the same individuals. The formaldehydogenic method of analysis with diffusion units is being used to check cortical hormone analyses obtained by the Talbot procedure. Preliminary results confirm the elevated excretion values. Ultra-violet absorption spectra show a qualitative difference between corticoid hormones excreted by cirrhotics and those excreted by normals. A large fraction of the corticoids excreted in cirrhosis retains the B-unsaturated			
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carbonyl grouping in ring A. Very low corticoid excretion values have been observed in obstructive jaundice. Five subjects were given pantoyltaurine, the structural antagonist of pantothenic acid, in an effort to produce a clinical deficiency of or to lower the urinary excretion of the vitamin.

The patients chosen were studied during a control period while on a general hospital diet adequate in vitamins; the 24-hour urinary excretion of pantothenic acid and pyridoxine was determined by a microbiological method. The individuals were then placed on a low B-vitamin diet containing approximately 3.0 mg of pantothenic acid and 1.5 mg of pyridoxine in 24 hours. This diet supplied approximately 1/3 of the optimum intake of the other B-vitamins. Increasing amounts of the potassium salt of pantoyltaurine, beginning with 150 mg, were given daily. This was dissolved in water and drunk by the patients on an empty stomach. The maximum amount given was 1 gm daily for 5 days. In addition each patient received 0.5 gm para-amino-benzoic acid daily and the percent acetylation of PABA in the urine was determined daily.

Five subjects were studied: the healthy individual mentioned above; 1 patient with cirrhosis; 1 with Wilson's disease (hepatolenticular degeneration); and 2 with nontropical sprue. Observation varied from 21 to 58 days, the normal individual being studied for the longest period. The minimal daily dose of pantoyltaurine was 60 mg and the maximal 1.0 gm. In no case was there a consistent depression of the urinary excretion of pantothenic acid. Values fluctuated considerably from day to day. Therefore an average value obtained from a short period of study was not considered reliable. The first table is an example of such a short-term experiment. The second table shows the results in a patient studied 58 days. No toxic effects of the substance were observed. Routine studies of the blood and urine showed normal values.

	(Average) Pantothenic acid in mg per 24 hours	(Average) Pyridoxine in mg. per 24 hours
Control period	1.608	0.839
Low B-vitamin diet	1.067	0.697
Low B-vitamin diet plus pantoyltaurine	0.867	0.488

	(Average) Pantothenic acid in mg per 24 hours	(Average) Pyridoxine in mg. per 24 hours
Control period	- - -	- - -
Low B-vitamin diet	1.16	0.138
Low B-vitamin diet plus pantoyltaurine	1.16	0.124

Since no effect from the oral administration of pantoyltaurine was observed, it was decided to give it intravenously. The patient selected was the 1 who had received 1.0 gm daily per os. To date amounts varying from 10 mg to 100 mg in a total volume of 30 cc normal saline have been given I.V. No immediate effects have been noted.

AFIP report not seen.

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Dr. Bang. Studies on the hepatitis virus included an attempt to obtain specific destruction of cells by the virus. To this end roller tube cultures of several human embryo (ca 4 mon.) tissues including liver, placenta, and intestine were grown in a completely homologous media for several months. They were grown both with and without clot in order to yield a high degree of exposure of the cells. Half of the cultures were inoculated with 2 drops of serum known to contain the hepatitis virus. The other half were kept as controls. Repeated observations during the next few months failed to indicate any specific cell destruction. Cords of liver cells remained intact. Cells were harvested for electron microscopy and are under study. To minimize the hazard of infecting technicians a vial type of roller tube with a special stopper was designed for future work. / An electron microscope study of sections of human liver has been undertaken with the thought that it permit seeing the virus in the infected tissue. To date we have studied biopsied specimens of 1 of each of the following conditions: normal liver, obstructive jaundice, and infectious hepatitis. These were useful for orientation but a great need is apparent for more such specimens.

Dr. Capps. Gamma globulin and immunity. The children in the orphanage continue to have infectious hepatitis. Difficulties have been met in obtaining sufficient normal children for study. At present it can only be stated that 0.01 cc gamma globulin per pound, given to individuals who are immediately exposed to presumably contagious cases, allows an altered mild form of the disease to develop. / Perhaps most important development during the past quarter has been the clear-cut demonstration of a fecal carrier state. Fecal material from 2 of our chronic cases, who have shown epidemiologic evidence of being carriers, has been fed to volunteers by Dr. Stokes and has produced infectious hepatitis. One child had been sick for 6 months, the other for 15. / Studies of a school epidemic of infectious hepatitis in children between 3 and 10 years in a Chicago suburb are almost completed. It is believed possible to demonstrate the importance of the unrecognized nonicteric case in transmitting the disease.

Dr. Davidson. Studies outlined in June 1951 have continued. These 2 have been completed: The effects of a purified and subsequently of an adequate diet upon hepatic function, size, and fat content (determined histologically by biopsy) were studied in 3 chronic alcoholics with fatty cirrhosis of the liver. None of the 3 showed significant improvement in hepatic function, size, or fatty content during 8 to 10 days of a purified diet consisting of glucose and minerals with the addition of thiamine alone in 1 patient and thiamine and multivitamins in another. (The thiamine was given because of co-existing Wernicke's syndrome which was also under study.) Following an adequate diet for 8 to 10 days, there was improvement in hepatic function in all 3 patients with a decrease in hepatic fat in 2. The 3rd patient did not show a significant decrease in hepatic fat until 15 days after the institution of an adequate diet, when a biopsy was taken. It was concluded that the improvement was related to the provision of an adequate diet and that little part was played by the withdrawal of alcohol or the rest in bed in the hospital. The effect of cation exchange resins in cirrhosis. Twelve patients with severe cirrhosis of the liver, ascites, and edema were observed clinically before, during, and after the administration of cation exchange resins (4 patients, ammonium resins, 4 patients, hydrogen resins; 4 patients, potassium resins) given as treatment for ascites and edema. During 15 courses of therapy of 30 to 90 grams daily in divided doses for from 3 to 13 days, 10 of the patients had a significant diuresis and reduction in ascites, edema, and loss in body weight. Neurologic disturbances developed on 8 occasions in 6 of the 8 patients with either of the resins containing ammonium. The disturbances consisted of drowsiness, apathy, the slurring of speech, disorientation, confusion, inappropriate behavior and a coarse, irregular, flapping tremor intensified by the sustenance of posture, and were noted as early as 3 days after instituting therapy. In 2 of the patients these changes occurred

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During the 1st course of resin, cleared promptly when the resin was discontinued and reappeared upon reinstitution of the resin. The tremor usually appeared first and lasted longer than the alterations in mental status and the changes were always fully reversible when the resins were stopped. The neurologic complications not associated independently with the serum potassium concentration and extent of acidosis so that they could not be attributed definitely to these abnormalities. In comparison, the patients given the hydrogen resins often had typical manifestations of electrolyte abnormalities. It was concluded that cation exchange resins are well tolerated well by patients with severe liver disease and should be administered cautiously. The use of resins does not at present replace the therapeutic benefit of an adequate diet rigidly restricted in sodium as the best method of management of ascites and edema in liver disease. The similarity of the tremor and changed mental status observed in the patients given ammonium containing resins to the neurologic manifestations of impending hepatic coma suggests possible common biochemical basis for these 2.

Dr. George. Antibiotics in Liver Damage. In addition to aureomycin and terramycin, penicillin, when given by mouth especially in form of its poorly soluble organic salts, has been found to be beneficial in delaying the onset of dietary hepatic necrosis in rats. Penicillin when injected was not or was only weakly effective. Among other antibiotics tested only neomycin showed some activity. Admixture of kaolin, kaolin, and pectin was ineffective. Tween 60 added to the diet (5%) had a definite delaying effect. The bacteriologic and metabolic studies on animals fed the necrogenic experimental diet with or without antibiotics are continuing.

Dr. Havens. MD 403. To test the ability of patients with viral hepatitis to increase circulating antitoxin following the inoculation of purified diphtheria toxin. Dr. Eichman has pursued a study at 98th General Hospital, Munich; 38 cases of hepatitis have been or are under study for antibody production. Of these 5 have received ACTH during the 1st 28 days of immunization; 1 has received ACTH for 14 days during the late stage of immunization; 1 was treated with cortisone for 14 days during the early stages of immunization and another during the 1st 28 days. The control group (16 orthopedic and general medical patients without known liver disease) have been included. MD 60. Viral Hepatitis and Hepatic Cirrhosis. In conjunction with studies on the effect of ACTH and cortisone on the capacity of patients with chronic hepatic disease to produce antibody, their effect on the serum proteins was investigated. In contrast to the diminution in globulin associated with the administration of ACTH and cortisone to patients with other diseases, in patients with chronic hepatic disease engaged in producing diphtheria antitoxin the diminishing effect was most irregular. In general, those who produced the greatest amounts of antibody had either little or no diminution or actually an increase in the amount of circulating globulin while receiving ACTH or cortisone, suggesting the possibility that the amounts of serum globulin and antitoxin found at any time during therapy with these substances represented the result of at least 2 opposing forces -- the specific stimulus for the production of antibody protein and a general inhibitory or catabolic action of ACTH or cortisone. Studies on the excretion of progesterone in the urine of patients with chronic hepatic disease following administration of progesterone (100 mg. daily for 3 days) have continued. Three normal controls excreted 12%, 13%, and 20%, respectively, and 1 case of rheumatoid arthritis excreted 45%. Two patients with chronic hepatic disease, well compensated and with only moderate functional impairment at the time of study, excreted 3%, while 1 with severe hepatic cirrhosis excreted 46%. Studies on the urinary excretion of 17-ketosteroids in patients with chronic hepatic disease following prolonged therapy with progesterone (100 mg. daily for 4 - 5 weeks) revealed an increase in 17-ketosteroids with a high free moiety, the latter finding similar to that following the injection of testosterone propionate in patients with hepatic disease. Also in progress are studies of the degree of inactivation of alpha-estradiol by slices

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and homogenates of normal and damaged rat liver during $\frac{1}{4}$ -, $\frac{1}{2}$ -, and 2-hour incubation periods. The residual estrogens are being partitioned into estradiol, estrone, and estriol fractions by the countercurrent distribution technique and are being determined by both bio-assay and fluorometric procedures. Preliminary data indicate that loss of biologic activity occurs rapidly, falling, in 7 instances, to an average of 14% of the original activity in 15 minutes, to 12% at 30 minutes, and to 4% at 2 hours. The corresponding values obtained by the fluorometric procedure were 56%, 39%, and 12%, respectively. Inasmuch as estradiol, estrone, and estriol, which differ greatly in biologic activity, yield much the same fluorescence values, the discrepancy observed between the results obtained by these 2 procedures may be due to conversion of estradiol to 1 or other of the less potent estrogens. This point will be clarified by partition studies.

Dr. Klatskin. Objectives are a) to investigate the relation of prolonged alcohol ingestion to the development of fatty infiltration and cirrhosis of the liver in experimental animals, and b) to study the factors which influence the course of Laennec's cirrhosis in man. This contract, initiated 15 June 1951, will amplify and extend observations made in this laboratory over more than a year. It was not possible to resume animal experiments until 15 September because of slow delivery of additional cages and animals. In the cirrhosis studies each subject requires at least 6 weeks' study in the hospital and at least 3 biopsies of the liver. These requirements limit the number of subjects available for study.

Relation of Alcohol Ingestion to Fatty Infiltration and Cirrhosis of the Liver in the Rat. Preliminary investigation showed that, under conditions of an adequate dietary intake of protein (casein 22%) and calories, alcohol increased the fat content of the liver, both when alcohol was permitted to raise the caloric intake over that of pair-fed controls, and when the intake was kept isocaloric with that of the controls by subtracting carbohydrate calories. Moreover, supplements of carbohydrate isocaloric with the alcohol intake of pair-fed controls failed to increase the fat content of the liver. These findings are consistent with the hypothesis of Best et al. that alcohol increases the choline requirement, but do not support their contention that this increment is the result of an augmented caloric intake. However, in preliminary experiments the increases in liver fat were very small although statistically significant, and the prophylactic effects of choline and methionine were not studied. Present experiments are designed to determine 1) whether alcohol increases the fat content of the liver independently of its caloric action when the protein intake is relatively low (12% casein); 2) whether choline and methionine prevent fatty infiltration under these conditions; and 3) whether alcohol increases the choline requirement by increasing fecal loss of nitrogen. Groups of rats (12 each) are being fed 11 diets. Complete weekly collections of feces are being analyzed for nitrogen content, and an attempt is being made to quantitate fecal trypsin to determine whether alcohol alters pancreatic function. At the end of 6 months the livers will be analyzed for their fat content and studied histologically. Factors Influencing the Course of Laennec's Cirrhosis. The purpose has been to analyze the relative importance of dietary factors, bed rest, and alcohol withdrawal on the clinical course. Initial studies showed that significant clinical, histologic, and functional improvement occurred when a suboptimal basal diet (1 gm of protein and 30 calories per kg of body weight without vitamin or lipotropic supplements) was administered for 4 to 6 weeks under controlled conditions of strict bed rest and alcohol withdrawal. When supplements of choline and, later, protein plus calories, were added, further improvement occurred but it was impossible to learn if this was due to the supplements or to the continued beneficial effects of the control regime. A 2nd group was studied under the same conditions, except that strict bed rest was not enforced and light exercise was encouraged. The results in this group did not differ significantly. At present choline is being administered during rather than

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following the control period, and biopsy and functional studies are being carried out at more frequent intervals to determine whether choline hastens or augments the rate of recovery.

Dr. Neefe. The incidence of abnormal hepatic tests in unselected blood donors and its relationship to the incidence of viral hepatitis in recipients. Period covered 1 January - 1 October 1951. Data obtained to date on volunteer blood donors at this hospital included these tests which were routinely used for classification of donors: 1'SB, TSB, Thymol Turb. (pH 7.8), Thymol Turb. (pH 7.55), Thymol Flocc. (2 tests), C24, C48, Zinc Turb., Urine (100 ml) Urob./ All donors are classified as to positive, negative, or questionable on the basis of these tests. "Scores" indicative of degree of positivity arbitrarily have been assigned to these tests. (The 2 thymol turbidity scores are averaged together for a single score. This procedure is also used in scoring the thymol flocc. tests. The assigned score of a CCF represents an average of the 24° and 48° scores). The total number of donors was 2090, of whom 187 were positive, 362 questionable and 1441 negative. Follow-up was possible on 51 positive and 14 questionable donors. The number remaining positive after subsequent follow-up: positive 15, questionable 6, total 21.

Analysis of frequency with which individual tests gave positive or questionable results in random screening of blood donors: Total number of "positive" donors, 187; number having only 1 test positive, 160; test most frequently giving positive results - TT, 99; Urine urobilinogen, 32./ The following represents the data collected to date on patients receiving blood from the above 2090 donors: The criteria used in selecting a recipient for future follow-up study: 1) Received blood from a positive or questionable donor. 2) Received a total of no more than 3 transfusions.// Statistics on acceptable recipients: Number receiving blood from positive donors, 69; number receiving blood from questionable donors, 65; number receiving blood from negative donors, 240; recipients disqualified because of receiving more than 3 transfusions, 292.

Number of those followed who have actually developed hepatitis, 0.

100 recipients who have received 3 or less transfusions of blood from negative donors are to be followed for control purposes.

Studies on Blood Donors Suspected as Carriers of Hepatitis Virus, because of development of Viral Hepatitis in Recipients include 12 patients who developed viral hepatitis after transfusion. The donors none of whom were included in the above donor survey, were then recalled for study, and 10 of them were classified as positive.// A 3rd phase comprises studies of sera from suspected carriers in volunteers in collaboration with Dr. Oliphant: a) sera from 5 suspected donors under test; b) each serum inoculated into 10 volunteers.

Dr. Oliphant. Inoculation studies have been initiated in 3 Federal prisons. Eighteen volunteers were inoculated with epidemic hepatitis culture material at Ashland prison on July 6. No cases of hepatitis have resulted; the study was terminated September 6. Sixty-four volunteers were inoculated at Lewisburg prison on August 2; 40 of these received pooled plasma which had been contaminated with specimens of serum and plasma from patients in the acute phase of serum hepatitis. Portions of this plasma were irradiated with ultraviolet light, under carefully controlled conditions, in an attempt to re-evaluate the efficacy of UV irradiation on the sterilization of plasma. Twenty-four individuals were inoculated at the same time with experimentally cultured serum hepatitis material. To date, no cases of hepatitis have appeared, but it is anticipated that cases may be seen during October. Sixty individuals were inoculated at McNeil Island prison August 24. Five groups of 10 each were inoculated with the sera of donors supplied by Dr. Neefe. The blood of these individuals had apparently induced serum hepatitis in the recipients, and the donors' bloods subsequently have been found to give abnormal results to 1 or more liver function tests. A 6th group of 10 was inoculated

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with human thrombin which had apparently induced serum hepatitis in patients at the Peter Bent Brigham Hospital.

Dr. Post. Nucleic Acids and Viral Hepatitis. Relatively little progress can be reported because this office did not receive the contract until 12 September. Studies are continuing on standardization of the chemical methods for the determination of tissue ribonucleic acid and deoxyribonucleic acid.

Dr. Reinhold. The results of red blood cell pyridine nucleotide determinations by the acetone conjugation method have been verified by a spectrophotometric method depending on sulfite reduction in a sufficient number of specimens to show that the 2 methods agree. This evidence provides further proof that the substance being measured and previously found to be elevated in the blood of patients with hepatic coma is pyridine nucleotide. However, neither method distinguishes between the several pyridine nucleotides that may be present. We are therefore preparing dehydrogenase to permit specific enzymatic reduction of diphosphopyridine nucleotide to make a differentiation possible./ Trials in which combinations of B vitamins, vitamin C, rutin, and liver stomach preparations were tested caused no change in red cell nucleotides in 2 normal males. This agrees with previous findings. A patient, markedly decompensated due to coronary artery disease, showed a high value for red cell pyridine nucleotide shortly before death. Hypoxia as a possible causative factor for high values will be further investigated./ Electrophoretic studies of serum albumin at acid pH made little progress during the summer due to high humidity and instrumental breakdowns. Efforts to define the optimal conditions for separation of the 2 albumin components of the serum of patients with liver disease has now been extended to the pH 5 region where different buffer combinations and ionic strengths will be tested./ Paper chromatography is being applied to the study of serum and urine of patients with severe liver disease, particularly those in hepatic coma, to detect and quantitate normal and abnormal components. The immediate concern has been identification as creatine and creatinine of the substances reacting with alkaline picrate. As mentioned previously, both may be increased in serum of patients with liver disease.

Dr. Stokes. The study of viral hepatitis in volunteers has been greatly facilitated by the formation of a Subcommittee on the Allocation of Volunteers, whose primary purpose is the interchange of information on human studies, the correlation of such work, the prevention, insofar as seems indicated, of duplication, and the joint use of common control groups wherever feasible. This Subcommittee has been active during the period of this report, with Dr. Stokes serving as chairman. The addition of a large group of volunteers in 3 Federal prisons under Dr. Cliphant and of a considerable number under Dr. Gordon in New York State will aid in a number of problems in a short time, as contrasted with several years' study in a single laboratory with a necessarily limited number of volunteers.

Infectious (Epidemic) Hepatitis. Growth of Virus. Attempts are being made to concentrate the virus from the infected amniotic fluid for complement fixation tests and the improvement of the yield for the starting material for skin tests. Skin tests. The lyophilized infected and irradiated amniotic fluid has proven somewhat more satisfactory for skin tests than previously in respect to stability./ An epidemic studied in Missouri by Dr. Drake permitted investigating the protective effect of a small dose of gamma globulin, .01 ml. per lb. body weight, and of the possible immunizing value of the skin test itself. The dosage of gamma globulin used has proven to be protective thus far. The data on the skin test are not complete, since the epidemic is still active. The correlation of positive history and positive skin tests was very close./ Carrier State. Stool preparations from 2 children in St. Vincent's orphanage who were suspected of being chronic carriers for 6 and 16 months respectively have been administered orally - 1 preparation from 1 child to 4 volunteers and a 2nd preparation from the 2nd child to another group of 4 volunteers - and have produced hepatitis with jaundice with a short incubation

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period in 2 volunteers, 1 from each group, and a case of hepatitis without jaundice in an additional volunteer. These results increase the value of the study done with Dr. Capps and add weight to the epidemiologic evidence for fecal-oral spread from children to student nurses over a period of many years. Further stools and serum from these children and others in the same orphanage will be tested.

Serum Hepatitis. Studies on the Virus. Previously amniotic fluid from the 9th passage of Ft. Bragg virus produced what appeared to be hepatitis without jaundice in volunteers. Blood serum from 1 of these volunteers (who received 9th amniotic passage) was in turn injected parenterally into 4 volunteers, 1 of whom developed hepatitis with jaundice and 1 hepatitis without jaundice with long incubation periods. It is possible (not probable) that the original seed virus may not have been diluted out sufficiently in 9 amniotic passages to indicate actual growth of the virus in the amniotic sac. Further amniotic passages are being studied.

Preparations from Convalescent Serum. Convalescent sera obtained from 18 S. H. cases who had had the disease 3 months to 3 years previously were pooled and processed, 1 part being freed of fibrinogen and thrombin by means of Kaolin. Gamma globulin from convalescents is being administered to 5 volunteers at the same time as infective serum (Ft. Bragg virus) and again 40 days later with a suitable group of 5 controls injected with the Ft. Bragg virus only. Under way is a similar study in which infective whole blood (Ft. Bragg virus) is being injected with and without the simultaneous parenteral injection of portions of the convalescent gamma globulin. The study is not complete but as of now the gamma globulin does not appear to have been protective. The preparation of serum has been delayed because of slight bacterial contamination, apparently from the Kaolin. This contamination has been eliminated and direct neutralization tests with this convalescent serum and the Ft. Bragg virus will be carried out for purposes of intravenous injection - a test which could not employ gamma globulin, since the latter cannot be injected intravenously. Through the Communicable Disease Center, Atlanta, Georgia, thrombin was obtained from a batch suspected of causing S. H. in about 11 neurosurgical cases operated upon at the Maine General Hospital, Portland, Maine. This material was prepared and injected subcutaneously in 4 volunteers. The results are not complete but 2 of these volunteers thus far have developed hepatitis without jaundice with an incubation period of 53 and 56 days, respectively.

Summary of article by Dr. Stokes et al., "Studies on the Agent of Infectious Hepatitis. IV. The Effect of Skin Tests for Infectious Hepatitis on the Incidence of the Disease during an outbreak in a Closed Institution:" The intradermal injection of infectious hepatitis skin test antigen may alter the susceptibility to the disease. At the onset of the epidemic about $\frac{1}{4}$ of the inmates were skin tested. During the next year 5 cases of hepatitis with jaundice were recorded among 320 skin-tested individuals with unknown histories, 1 in the 144 skin-test-positive, and in the 176 skin-test-negative subjects. In contrast, 112 cases occurred among the 825 non-skin-tested individuals. Thus the incidence of jaundice in the skin-tested group was 1.6 % as against 13.6% in the non-skin-tested individuals. Possible explanations are discussed.

Dr. Turner. The possible use of dextran as a nonantigenic colloid in the differential flotation of lipoproteins in order to get purified antigens for our immunochemical studies has been explored. A grant from AEC to study effects of radiation injury in patients receiving therapeutic doses of radiation will be devoted to 2 phases: an intensive study of the changes in lipid patterns as determined by our ultracentrifuge technique, and a clinical laboratory study of liver function in a somewhat larger group. These studies are interrelated with those of AFEB. A Spinco ultracentrifuge for both analytical and quantity techniques will be set up in this laboratory.

g. Reports. "The Study of Serum Proteins and Lipids with the aid of the Quantity Ultracentrifuge," (accepted by J. Clin. Invest.); Paper from 10th Conference on Liver Injury to be published ~~RESTRICTED~~ 84 Page 8 of 16

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Walter Reed Army Medical Center. Purpose: To determine the incidence of hepatitis following the use of irradiated plasma among Korean casualties./

All Korean casualties admitted to 10 selected wards at Walter Reed Army Hospital are screened to determine how many received plasma transfusions. These wards include General Surgery, Neurosurgery, and Orthopedic Surgery. All patients who are known to have received plasma or in whom plasma transfusion is highly likely are followed, and examined weekly by a Department physician. Key questions and points to be noted in the physical examination have been laid out on a work sheet for each patient which can cover 4 months. Where the findings are questionable as regards hepatitis, blood samples may be drawn and liver function tests performed. In addition, morning urines are being collected every other day on 5 selected wards, and urine bilirubin and urobilinogen measurements are made. To date 4 cases of hepatitis have been detected by this additional survey tool at a time when detection was not possible by other means.

Since 1 March 1951 this survey has also included Valley Forge and Percy Jones Army Hospitals. Four hundred Korean casualties have been surveyed at Walter Reed Army Hospital to date. Approximately 1/2 of them have received plasma. Because of extensive wounds and the great amount of blood administered in the remaining half, these patients are also being followed./

Period September 1950 to June 1951. In patients who received plasma plus blood the incidence of hepatitis is 21.6 per cent. In those who received blood alone the incidence of hepatitis is 3.6 per cent. "Hepatitis" as used here, 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 as long as fresh Korean casualties continue to be admitted to the 3 hospitals.

Acute and Chronic Diseases of the Liver. Relations of Serum Iron. Clinical significance of serum iron levels in the differential diagnosis of liver disease (June report) has been confirmed by additional cases. A more sensitive reagent for iron determinations has been studied and successfully used for routine analyses./ Although Fe^{59} remains unavailable for human use, preliminary studies on the separation of the cupferron iron complex from tissues followed by a micro-electrodeposition technique indicated the basis for a successful microanalysis of radioiron. Such a method is in demand because there has been no reliable method published for separating traces of iron from large amounts of organic material (animal carcass, feces, urine) prior to electroplating. If this proves successful, it should very substantially advance investigation of iron metabolism./ The 1st phase of the project on iron metabolism in experimental liver disease has concerned the study of iron transport in the intestinal lymph of the rat. To date it indicates that no iron is transported through the intestinal lymph of rats.

g. Reports. "The Serum Iron in Acute Hepatitis," Peterson, Ralph, J. Lab. & Clin. Med. (in press).

"Iron Transport in the Intestinal Lymph of Rats," Peterson, Ralph (in preparation).

Iron Metabolism in Liver Disease. Ultimate purpose: To employ Fe^{59} for study of patients with acute hepatitis. No radioactive iron suitable for human use is now available. Techniques for the determination of radioactive iron have been set up. To gain experience, animal experiments are contemplated, using Fe^{55} plus Fe^{59} mixtures./ The 1st may be completed in 1 month. It relates to the route of iron absorption and the transport of iron in the lymph. In rats with total intestinal lymph fistulae, less than 1 per cent of a dose of radio iron

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Injected parenterally appeared in the lymph; the rate of disappearance was approximately the same as blood disappearance rates. When the animals were given the radio iron orally as ferric ammonium oxalate, ferric citrate, and ferrous ascorbate, a very small percentage of the iron appeared in the lymph (less than 0.1%), whereas 2-4% of the total dose was absorbed from the gastrointestinal tract. Studies indicate that iron is absorbed primarily into the blood stream.

The Relationship of Alloxan to Human Metabolic Processes. Previous studies revealed the possibility that humans excrete up to 100 mg or more of an alloxan-like derivative, indicating possibly a new metabolic pathway for purines and/or pyrimidines. Presumably this metabolic process takes place in the liver. Purpose of this study is to determine whether 1) alloxan is the processor of oxomalonic acid isolated in the urine, 2) whether patients with liver disease excrete more or less of this obscure compound, and 3) whether a previously unrecognized metabolic process is present in the liver.

Method. Half the molecule of alloxan is oxomalonic acid, the other half is urea. A compound in urine can be isolated which on hydrolysis yields oxomalonic acid. The latter can be isolated and measured by using paper chromatography. With the aid of paper chromatography, ion exchange, silica gel chromatography, and counter current distribution, it seems possible to identify the compound in urine which yields oxomalonic acid. This compound will be measured in the urines of normals and persons with liver disease.

Total Circulating Albumin in Infectious Hepatitis. Iodinated human albumin (I^{131}) will be used to estimate the albumin distribution department by the isotopic dilution technique. Thirty to 40 mc of labeled albumin will be given intravenously at each test. Periodic samples will be drawn and the counts per cc will be plotted on semilog paper. Sensitivity of the isotope method will be assessed by injecting known amounts of unlabeled serum albumin and repeating the estimation immediately thereafter.

ACTH Therapy in Acute Viral Hepatitis. ACTH is administered intravenously throughout the entire course until the total serum bilirubin returns to normal limits. Patients are selected on an alternate basis and the controls given an intravenous drip of glucose resembling ACTH. They are followed clinically and by liver function tests and liver biopsy. Progress has been slow because suitable cases have not been made available. The results at the moment show that the patient receiving ACTH will do better by every standard than will a control. When comparing the duration of time required for the various liver function tests to return to normal from the onset of jaundice in the controls and treated cases, the differences are not statistically significant.

Ultramicroprecipitation. Purpose. To develop a filter paper electrophoresis method in order to allow chemical studies of the variation of proteins in liver disease. Serum is applied to filter paper which is wetted with the desired buffer and then placed in an electric field. After 3 hours the paper is dried in hot air, fixed in Hayem's solution, and stained in bromphenol blue. The protein fractions, albumin, alpha, beta, and gamma globulins are clearly separated and stained blue. Total nitrogen by a new ultramicro Kjeldahl method is measured on each protein fraction. Data are being accumulated and comparisons are being made with the usual methods for protein fractionations. It is hoped that when this method is standardized it can be used on a routine basis.

Production of Hepatic Coma in Dogs. Purpose. The availability of a dog preparation for the study of hepatic failure would facilitate fundamental investigation of hepatic physiology with reference to therapeutic trials.

Hepatic coma in dogs will be attempted by ligation of the common hepatic

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artery 24-48 hours after production of a complete ECK fistula. Therapeutic measures will be evaluated, and intermediate carbohydrate metabolites and the value of the artificial kidney will be studied./ One dog died following the 2d stage. Autopsy was done.

Hepatic artery - portal vein anastomosis, was attempted in 5 dogs. In all but 1 the anastomosis thrombosed (on the arterial side). In 1 dog a thrill was palpable at the end of 1 month but no longer present when re-operated 4 months later. Liver biopsies done at a time when the anastomoses were occluded showed changes similar to those described following simple hepatic artery ligation (i.e., central necrosis and cellular infiltration). This phase has been discontinued.

In Vivo Testing of the Citric Acid Cycle in Man and Animals: under various conditions of loading with metabolic precursors and intermediates. Methods. 1) A method for alpha-ketoglutaric and pyruvic acid measurement is available; 2) measurements of the various anion fractions of blood and urine, including particularly the organic acid fraction; 3) attempts to directly study the organic acid fraction with paper chromatography; and 4) patients and animals will be treated with components of the citric acid cycle and the effect on the cycle will be tested by studying the concentrations in blood and urine of the keto acids, the organic acids as a group, and individual organic acids by means of paper chromatography.

Portal Circulation Time as an index of portal hypertension. Using the technique of Newman & Cohen, vaporized ether is instilled rectally; time required to appear in the expired air is measured. In the presence of a normal systemic circulation time, prolongation of this interval is believed to represent a slowing of portal blood flow and an increase in portal venous pressure./ These measurements are part of a clinical study of porto-caval anastomoses in patients with portal hypertension. Normal values range up to 25 seconds. In 4 patients with portal hypertension and esophageal varices, the values were elevated. In cirrhotic patients without other evidences of hypertension normal values are obtained.

Characterization of Pathological Hepato-renal Interrelationships.

Purpose. To develop a dog-preparation in which liver failure precedes renal failure and to study the metabolic changes which characterize the picture, with a view to reversing these changes./ Dogs are subjected to common bile duct ligation and cholecystectomy. The obstruction is later relieved by means of external biliary fistula in an attempt to reproduce the coma demonstrated by Boyce and McFetridge. The clinical course following the production of this coma will be investigated by means of urinalysis, anion fractionation, record of urine volume and fluid intake, alpha-ketoglutarate levels, electrolytes levels, BUN, and creatinine. The method of Rappaport for the production of hepatic coma is now to be studied. Four dogs have been subjected to total CBD ligation with subsequent sudden release of the obstruction, utilizing the method of Boyce and McFetridge. No hepatic coma resulted. Micro sections of liver at autopsy (animals sacrificed) reveal little abnormality; sections of kidney reveal occasional bile casts in tubules without evident change in glomerular or tubular structure.

Urinary Steroid Excretion in Hepatic Disease. Daily 24-hour urine collections are made from patients studied, and steroid levels determined under various conditions of therapy. Hormone excretion will be measured in those receiving ACTH and cortisone./ A large number of determinations have now been made. These have been performed in patients with hepatic cirrhosis, with obstructive jaundice, and with acute and chronic viral hepatitis. In the latter 2 groups studies have also been made under the injections of ACTH therapy. In general with the methods available, patients with hepatic cirrhosis and chronic hepatitis show slight elevations above the normal of urinary corticoids. Patients with acute hepatitis, on the other hand, show normal values. The 17-ketosteroid excretion in chronic hepatitis

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and cirrhosis was within normal limits. The 17-ketosteroid excretion in early acute hepatitis was low, with a slow return to normal paralleling clinical improvement in the disease. In patients with acute and chronic viral hepatitis who are given ACTH there is a corresponding elevation of urinary corticoid excretion. This elevation is directly proportional to the dose of ACTH used. The 17-ketosteroids in these patients is apparently unaffected by intravenous ACTH. This latter observation may be related to the fact that the method used allegedly does not measure dehydroisandrosterone which is the androgen thought to be derived from adrenal cortical hormones. Studies are in progress to determine the concentration of this latter hormone, and also to determine differences, if any, in the corticoid excretion when glucuronidase hydrolysis rather than acid hydrolysis is used.

Albumin Binding of Bromsulfalein (BSP) dye. Purpose. To study the binding of BSP dye in liver disease, and to determine the value of a colorimetric calculation of serum albumin based upon the ability of the albumin to decolorize an alkaline BSP solution./ Using a standard BSP solution buffered to a pH of 8.2, a curve was constructed plotting concentration of albumin and percent transmission. With this as a standard, serum is then analyzed in place of the known albumin solutions./ Technical difficulties have delayed accumulation of data. In a preliminary way the concentration of albumin determined chemically parallels roughly that calculated with this method except at low concentrations of albumin.

Hepatic Vein Catheterization permits the measurement of hepatic blood flow and provides blood samples from the hepatic vein for other metabolic measurements. 1) The effects of certain drugs on hepatic blood flow are determined by comparing the results before and after intravenous administration. Adrenaline and histamine have been studied. Norepinephrine and 1 of the parasympathomimetic drugs will be investigated. To study their effects on the venous sphincter mechanism these experiments will be repeated on cats (the sphincter mechanism of cats more closely resembles that of humans)./ 2) The oxygen content and saturation of hepatic vein blood in normal dogs has been measured. One patient with hepatic cirrhosis has been studied./ 3) Hepatic vein blood has been collected following infusions of fructose in 1 dog and 1 human and was analyzed for carbohydrate and carbohydrate intermediates.

Results. 1) In early experiments it appeared that adrenalin produced an increase in hepatic blood flow as measured by the BSP method. Subsequent experiments, in which the peripheral concentration of dye exceeded that in the hepatic vein, suggested that this effect was more apparent than real. Because adrenalin (0.3 - 0.5 cc of 1:1000 solution) appears to have a direct effect on BSP extraction, this method seems unsuitable for determining hepatic blood flow. These tests will be repeated with smaller amounts of drug. Histamine appeared to reduce the calculated hepatic blood flow but the results may be similarly criticized. Additional observations are necessary./ 2) The oxygen saturation of the hepatic vein of 1 patient with cirrhosis was measured as 70%, while that in the portal vein was 80%. The difference in O₂ content which this represents is 1.5 vols %./ 3) The results of 3) above appear in another progress report. In view of the lack of sensitivity of the prothrombin time determination, no difference could be detected in hepatic and peripheral vein blood.

Glucuronic Acid Metabolism. Purpose. To study glucuronic acid formation, conjugation, and excretion in normals and in patients with acute and chronic liver disease in the resting state and in response to specific substances which the liver inactivates by conjugation with glucuronic acid. Methods. The carbazole method of Dische is used to measure a) glucuronic acid excreted in the urine of normals and patients with acute and chronic liver disease./ b) glucuronic acid

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excreted in similar subjects in response to specific materials, such as benzoic acid, which are detoxified by glucuronic acid conjugation. Fishman's method will be used to study serum B-glucuronidase levels in these groups.

The chemical procedures of Dische and Fishman have been set up and standardized. Nine 24-hour glucuronic acid determinations have been made in 5 normals, 6 determinations in 1 patient with acute hepatitis, and 6 in 1 patient with portal cirrhosis. Conclusions cannot be drawn from these data.

Anion Fractionation of Urine. Purpose. To elucidate the handling of certain electrolytes by patients with acute and chronic liver injury and related metabolic conditions and to study the excretion of organic acids in metabolic diseases.

A strong cationic resin is used to remove cations from urine, leaving the anions in their acid form. The urine is then titrated to give total anions (minus bicarbonate). An aliquot of urine is treated with barium hydroxide to precipitate mainly phosphates and sulfates. This mixture is put through the cationic column and the effluent urine titrated. This measures total anions minus phosphates, sulfates, and barium insoluble acids. Chlorides are measured in this barium filtrate. The barium filtrate anions minus chlorides equals the organic acid fraction of urine. A portion of the barium filtrate is evaporated to dryness and the acidity and chloride content of the residue is determined. In this way the organic acid fraction is divided into volatile and nonvolatile acids. The method has been tested by adding to urine measured amounts of the following: chloride, phosphate, sulfate, citrate, and acetate. Each compound has been accurately detected in the fraction to which it theoretically belongs. The method as applied to urine works well. It is being applied to blood filtrates with less success. It appears that the anion fraction of plasma is larger than that now considered. This will require further study. A micro-modification has been developed which allows studies on 1 cubic centimeter of urine or plasma. Clinical data are being accumulated on patients with acute hepatitis, various infections, and metabolic diseases.

Determination of Alpha-ketoglutaric Acid and Pyruvic Acid in Liver Disease.

It has been reported that pyruvic acid in blood of patients with hepatic coma is elevated. A more specific method for pyruvic and alpha-ketoglutaric acids is now available. These acids represent important intermediates in carbohydrate and protein metabolism, the blood levels of which might reflect certain metabolic aberrations. Studies of these keto acids in liver diseases are being arranged. The keto acids are converted to their 2,4 dinitro-phenylhydrazones and these are fractionated and isolated on paper chromatograms. The isolated keto acid derivatives are readily measured colorimetrically. Precise and specific measurements are possible for the first time. Studies of severe liver disease continue to show marked elevation of alpha-ketoglutaric and pyruvic acids. In severe cirrhosis alpha-ketoglutaric acid in the blood remains consistently elevated. More clinical data are being accumulated. The carbohydrate intermediates are being followed during glucose and fructose tolerance tests in various clinical situations.

Histochemical Studies of Liver Alkaline Phosphatase. This study is designed to clarify whether the elevated phosphatase in obstructive jaundice is of hepatic or extrahepatic origin. Young rats are joined in parabiotic union and the common bile duct of 1 is ligated. The unobstructed biliary system clears the products of biliary retention from both animals. If under these circumstances the liver of the obstructed animal can be shown to contain an excess of alkaline phosphatase, there would be evidence for the hepatic origin of the enzyme. To date 1 pair has been biopsied and abundant alkaline phosphatase has been demonstrated in the obstructed liver.

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Bromsulfalein Metabolism. Dogs and rats are prepared by 1) ligation of the common bile ducts. BSP dye is injected postoperatively and the duration of retention is determined by daily blood samples./ 2) to determine the effects of aureomycin on the removal of BSP by the liver simultaneous intravenous infusions of the antibiotic are administered to dogs and the BSP extraction determined by analyzing hepatic and peripheral vein blood./ 3) studies are made of the BSP recovered from urine and feces of normal dogs and those with obstructive jaundice. These studies have been extended to human subjects.

a) In contrast to the results in humans, prolonged retention of BSP was not observed in dogs and rats with experimental obstructive jaundice. Analysis of blood samples drawn 72 hours after injection of dye showed no significant concentration of BSP in any animals studied. This suggests that these animals handle BSP differently than do humans. b) An intravenous infusion of aureomycin (100 mgs given in 10 minutes) had no effect on the extraction of BSP by the liver. This is to be expected, since the uptake of dye by the liver is not controlled by an enzymatic process. If aureomycin does have a direct effect on BSP retention it is anticipated that its action would be primarily on the excretion of BSP into the bile, inasmuch as this latter process is probably an enzymatic one. c) Depending on the route of administration of BSP in dogs or humans, the percentage of dye recovered from the urine and feces varies considerably. In normal dogs 5-10% of the amount given is recovered after intravenous administration, and 20-30% is recovered if it is given orally. In dogs with obstructive jaundice, on the other hand, 45-55% of the dye is recovered following I.V. injection, whereas only 1-10% is found when given orally. A somewhat smaller amount of dye (15-25%) is recovered from the feces of normal humans when given orally as compared to dogs. In liver disease, over 55% of dye given is found in the feces (50-60%) and urine (2-8%).

Fructose Metabolism in Hepatic Disease. Purpose. To compare the relative rates and completeness of the utilization of fructose and glucose administered alone or together as invert sugar in various stages of liver disease and diabetes. Intravenous infusions of glucose and/or fructose are given at 0.5 gms per kilogram for 30 minutes to normals and to patients with hepatitis, cirrhosis, fatty liver, and diabetes. Blood samples drawn at frequent intervals over a 2-hour period are measured for glucose by the Nelson method, fructose by the Roe method, inorganic phosphorus by the Fiske & Subbarow method, serum potassium by the standard flame photometer technique, lactic acid by the Summerson method, and alpha-ketoglutaric and pyruvic acids by paper chromatography. Studies on the effects of constant infusions of glucose, fructose, and invert sugar are also in progress. Such techniques are being applied to dogs and patients with cirrhosis, using the hepatic vein catheterization techniques to obtain hepatic vein blood.

Over 100 carbohydrate tolerance tests have been done in normals or in patients having liver disease or diabetes. Normal standards for glucose, fructose, and intermediary metabolites have been determined. Patients with acute hepatitis and cirrhosis of the liver show normal fructose utilization in the presence of abnormal glucose tolerance. In diabetes mellitus, fructose utilization appears to be only slightly delayed in the presence of markedly altered glucose utilization. The blood levels of metabolic intermediates are much higher in normals and in patients with liver disease and diabetes following fructose than following glucose administration.

This study is necessary to aid in the interpretation of the histochemistry of human livers and to permit the selection of appropriate stains./ In addition to human biopsy material, animals will be subjected to a variety of hepatotoxins, allopotrophic diets, and surgical procedures. AFIP is collaborating. These stains are now available: alkaline phosphatase, esterase, lipase, and desoxyribonuclease.

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In the near future these will be: sulfhydryl group, cholinesterase, hyaluronidase, ribonucleic acid, and acid phosphatase.

AFIP has improved the quality of several of their stains and it is believed they are ready to be applied to selected human liver biopsy specimens.

Dr. Watson. Clinical studies of the therapy of chronic liver disease with reference to porphyrin metabolism are continuing. Studies with riboflavin, phosphate, co-carboxylase, nicotinamide, crystalline B₁₂, and combinations; of lipo-adrenal extract; and of testosterone propionate individually ~~have~~ ^{have} been carried out in an effort to determine whether they have any sharp effect on urinary coproporphyrin excretion or on other disturbed liver function tests. There has been no significant evidence of benefit, but the studies are not complete. There is some evidence that a combination of substances needed for cellular metabolism, together with aureomycin, may be truly beneficial in severe hepatic insufficiency, with or without coma.

Further information is being accumulated on porphyrin metabolism in liver disease and jaundice. It has been established that normal serum does not contain any coproporphyrin. In accordance with reports of van den Bergh, significant amounts are often encountered in patients with jaundice or liver disease. Results here are inconclusive as yet as to whether there is any significantly greater increase in patients with parenchymal liver disease and with extra-hepatic biliary obstruction. Other questions are: the frequency and extent of increases of the serum coproporphyrin in liver disease without jaundice./

Studies with Miss Hawkinson have confirmed fully the preliminary report that porphobilinogen is separable from a uroporphyrin chromogen which does not give the Ehrlich aldehyde reaction. Once separated from this chromogen, porphobilinogen on heating does not yield porphyrin, but only undergoes change to the dark brown pigment porphobilin. These results have considerable significance; it now appears likely that porphobilinogen is fabricated in the liver, rather than in the bone marrow. Studies with Dr. Schmid, supported ~~partly~~ ^{fully} by AEC, in cases of porphyria of the intermittent acute, or of the mixed type showed that the liver contains relatively large amounts of porphyrin while the concentration in the bone marrow is normal. This was particularly striking in cases of the mixed type in which liver functional impairment is most often seen.

A paper to appear in August, Journal of Laboratory and Clinical Medicine, describes work in which the fractional serum bilirubin has been studied intensively with reference to the clinical value of the prompt direct reacting or 1-minute fraction. This work has fully confirmed and extended our previous observation of usefulness of determining this fraction, but has not thrown any additional light on its fundamental significance.

The dextrorotatory urobilin of the feces and urine. Information thus far obtained indicates that all or nearly all of the levorotatory stercobilin is extracted by petroleum ether from the primary ferrous hydroxide filtrate (after acidification) in the quantitative procedure, but that ethyl acetate extracts a further urobilinoid pigment which is dextrorotatory. This study does not yet permit any definite conclusion, but it appears to be of considerable importance. The application of a very sensitive microfluorospectrophotometer to the problem of liver cytochemistry is being investigated with Dr. Schwartz and others.

Instrument Development (in brief): Calibration curves were prepared to permit interpretation of the recorded spectra and to determine the degree of resolution obtainable. A new lamp housing has been designed to permit more intense and uniform illumination of the microscopic field.

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Monochromator. A Bausch and Lomb monochromator borrowed from the Department of Chemistry is being used to permit selection of specific wave lengths for excitation of fluorescence. A unit designed for the liquid air cooling of tissue sections and small volumes of solution is nearing completion. Delivery is awaited on an extremely sensitive photomultiplier tube manufactured in England which is expected will permit a considerable increase in sensitivity over the present IP-21 tube.

Recording mechanism. A selsyn remote-control drive assembly is being substituted for our former mechanically coupled gear train reversing clutch. A vertical illumination microscope (metallurgical microscope) has been found to offer many advantages over the usual type of microscope and will be used for most future fluorescence microscopy studies. A Glick type freezing drying unit has been completed.

Experimental studies under Dr. Hoffbauer dealt with 2 problems: 1) dietary-induced liver necrosis in rats with reference to possible protective substances and the production of postnecrotic scarring; 2) attempts to adapt the virus of human hepatitis to an experimental animal.

1) (a) A liquid polyamine methylene resin with silicates ("Resion") was added to the daily ration of animals receiving a necrogenic diet. The average survival for both the control animals (11) and the treated group (8) was 38 days. When repeated in 17 treated and 17 control animals, the average survival time was 36.5 and 41.5 days, respectively. Conclusion: the addition of this agent, in a daily dose of 1 cc., does not influence the course of dietary liver necrosis./

(b) A group of 36 male rats weighing 50 grams were placed on the necrogenic yeast diet. Starting on the 20th day, daily urine bilirubin tests were performed on each. The animals survived from 27 to 71 days; 11 animals exhibited intermittent bilirubinuria for 5 to 32 days prior to death. Ten of these showed gross evidence of postnecrotic scarring. It appears that liver necrosis, under current conditions of production, is not uniformly fatal with the 1st attack. The ability to detect such attacks permits a study of various therapeutic agents as well as of the natural course of experimentally-induced coarsely nodular cirrhosis.

2) Attempts to adapt the virus of hepatitis to hamsters and rats by administering cortisone prior to infection, while unsuccessful to date, are continuing. Rats 7 to 14 days of age were given 5 mg of cortisone, followed in 48 hours by a similar dose. Infective serum was injected intraperitoneally at that time. At 21 to 28 days of age the animals were placed on a yeast diet fortified with the minimum amount of cystine and tocopherol necessary to prevent spontaneous necrosis of the liver. Observations have included urine tests for bilirubin and the histologic examination of the liver on animals sacrificed at intervals. All results to date have been negative; investigations, including attempts at serial passage, are continuing.

Reports. Watson et al.: "Porphyrin Chromogens or Precursors in Urine, Blood, Bile, and Feces," and "An Improved Method for the Determination of Urinary Coproporphyrin and an Evaluation of Factors influencing the Analysis," J. Lab. & Clin. Med., 37:831-859, June 1951.

Gyorgy, Stokes, and Goldblatt: "Antimicrobial Agents in the Prevention of Experimental Dietary Injury of the Liver," Trans. Assn. Amer. Phys., 64: 289, 1951.

Zieve...and Watson: "Normal and Abnormal Variations and Clinical Significance of the One-minute and Total Serum Bilirubin Determinations," J. Lab. & Clin. Med., 38:446-469, Sept., 1951.

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Dr. Kinsell
Highland Hospital

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SECURITY CLASSIFICATION
PROGRESS REPORT (QUARTERLY)

RESEARCH AND DEVELOPMENT PROJECT CARD (NEW PROJECTS) 2. SEC.		3. PROJ. NO. 6-60-11-16	
1. PROJECT TITLE Nutrition in Disease and Convalescence		4. REPORT DATE 30 Sep 51	
6. BASIC FIELD OR SUBJECT		7. SUB FIELD OR SUBJECT SUB GROUP	
8. COGNIZANT AGENCY	12. CONTRACTOR AND/OR LABORATORY		CONTRACT/W. O. NO.
9. DIRECTING AGENCY			
10. REQUESTING AGENCY	13. RELATED PROJECTS		17. EST. COMPL. DATES
11. PARTICIPATION AND/OR COORDINATION	14. DATE APPROVED		RES.
	15. PRIORITY		DEV.
	16.		TEST
19.		OP. EVAL.	
		Fy 18. FISCAL EST' S.	
20. REQUIREMENT AND/OR JUSTIFICATION			
21. BRIEF OF PROJECT AND OBJECTIVE			
<p>Work undertaken: 1) Further studies of the nature of the metabolic defect in 2 patients with idiopathic hypoproteinemia, using S³⁵ labeled methionine./ 2) Evaluation of the metabolism of inorganic sulfate (using S³⁵ labeled sulfate) in human subjects./ 3) Evaluation of the protein metabolic defect in a patient with long-standing cystinuria. This laboratory has made use of S³⁵ labeled methionine, S³⁵ labeled cystine technique, and microbiologic assay of cystine and methionine. Work with Dr. Harold Tarver has included the quantitative and qualitative determination of multiple amino acids in the urine, and has shown the presence of large amounts of amino acids, other than cystine, in the urine./ 4) The use of ACTH as an index of the rate of wound healing.</p> <p>Two conclusions are drawn: 1) The uptake of S³⁵ labeled inorganic sulfate in experimental wounds in rats with any technique so far used has been too low to permit of evaluation./ 2) The rate of uptake in rats receiving S³⁵ labeled methionine is very significantly higher in experimental wounds as compared to areas of normal skin. This technique is in process of standardization.</p> <p>Plans include standardization of the S³⁵ method for evaluation of the rate of wound healing, and determination of the effect of various agents administered systemically and locally upon the rate of wound healing using the technique above noted. Among these agents will be anabolic steroid hormones, cortisone-like adrenal steroids, and certain dietary factors.</p> <p>This project assigned new number - 6-60-11-17 - on 8 October 1951.</p>			
115			
22. JRDB	SN.	PC.	IC & P.
			X. I. C.

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AFIP, AMSCS (2), Avery,
Becks, Bibby, Boyd, PROGRESS REPORT (QUARTERLY)

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RESEARCH AND DEVELOPMENT/PROJECT CARD/NEW PROJECTS		2. SEC.	3. PROJ. NO.	6-63-01-6
1. PROJECT TITLE Oral Disease		4. REPORT DATE 30 Sep 51		
6. BASIC FIELD OR SUBJECT		7. SUB FIELD OR SUBJECT SUB GROUP		
8. COGNIZANT AGENCY	12. CONTRACTOR AND/OR LABORATORY		CONTRACT/W. O. NO.	
9. DIRECTING AGENCY				
10. REQUESTING AGENCY	13. RELATED PROJECTS		17. EST. COMPL. DATES	
11. PARTICIPATION AND/OR COORDINATION	14. DATE APPROVED		RES.	
	15. PRIORITY		DEV.	
	16.		TEST	
19.		18. FISCAL EST'S.		
20. REQUIREMENT AND/OR JUSTIFICATION Day, Engel, M., Goldman, Hollenback, Kerr, Kruger, Mann, Marshall-Day, NBS, Percy Jones Army Hospital, Schour, Wainwright, Weismann.				
21. BRIEF OF PROJECT AND OBJECTIVE <u>AMSCS. Cephalometric Studies:</u> The Cephalometer as an Adjunct to Surgical Procedures. The precision of cephalometric methods has resulted in the development of: a) means of detecting foreign bodies (where other methods proved inadequate); b) precise location of the plane of resection in sliding osteotomies; c) design of prosthesis replacing resected cancerous facial bones./ <u>Röntgenographic Cephalometric Appraisal of Craniofacial Dysplasias.</u> The original intention of evaluating results of surgery has advanced to evaluating the nature of form and function preparatory to operative procedures. <u>Assessment of the Mechanics of Orthodontic Therapy.</u> The present number of cases is twice that of the preceding period. The 1st group are now being run in series. The limitations and possibilities of this method/treatment are becoming more evident. <u>Prosthodontics. Movements and Positions of the Tongue.</u> Progress has been made in developing x-ray procedures, exposure factors, barium paste use, and an initial view of subjects. Test runs have been completed and surveys begun. <u>Periodontics. Cephalometric Appraisal of Occlusal Equilibrium.</u> Compilation of material is progressing. <u>Cephalometrics: The Adult Face; Significance of a Complete Dentition.</u> The minimum numerical case requirement for anthropologic acceptance in normal adult face study has been reached. An evident similarity of facial characteristics, suggestive of type forms, has been recognized. The form and relation of palate, tongue, and lip structures bear note./ <u>Temporomandibular Joint Disturbances</u> (newly initiated project). Cephalometric procedures have been emphasized in the treatment of these cases. Over 36 cases have been analyzed and treatment carried out or recommended. Evaluation will follow./ <u>Cleft Palate Clinic Studies</u> (new) will cover 1) time and method of treatment, 2) nature of surgical repairs, 3) speech evaluation from roentgenographic readings.				
22. JRDB	SN.	PC.	IC & P.	X. I. C.

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AMSGS - Dental Caries. Environmental Factors Influencing Acidogenesis in the Oral Cavity and in the Carious Lesion. Determination of the respiration rate of the saliva of caries-susceptible and caries-free individuals has been attempted in order to correlate this action with freedom from or susceptibility to caries. To date 17 individuals have been tested and this will continue until sufficient data have accumulated to permit valid conclusions. Studies have also been made of the relationship of the rate of respiration of saliva and its acid-producing capacity in the presence of various carbohydrates. They indicated that the oral bacterial flora produce as much acid from polysaccharides as from the simpler sugars. A direct correlation exists between respiration rate and the acid-producing capacity of saliva./ Chemical Composition of the Organic Matrices of Dentine and Enamel. A micro and ultra-micro Kjeldahl method for the estimation of nitrogen has been set up. The effects of various buffered acids on the nitrogen, phosphorus, and calcium content of sections of dentine, which have or have not been treated with various fluorides, are being studied. A series of 21 naturally occurring amino acids thought present in the organic matrix of tooth structure have been subjected to partition chromatography under varying conditions in order to have a known reference for future work. Tooth samples are being prepared for hydrolysis after which they will be subjected to partition chromatography./ The Analysis of the Inorganic Components of Enamel and Dentine. The teeth of some 20 hamsters have been collected for chemical analysis of the enamel and dentine. Using the flotation method, the enamel and dentine have been separated and isolated as pure tissues and analyzed for calcium and phosphorus. The analysis for magnesium, organic content (by weight), and moisture content will follow. A mass spectrophotographic analysis of samples of enamel and dentine for trace elements will be completed shortly. The extracted organic matter from enamel and dentine will be subjected to chromatographic analysis to determine more exactly the composition of the organic phase. A similar investigation will be made of teeth of various species of monkey in the hope of finding a suitable animal for caries experiments./ Caries in animals and Humans following Irradiation. Further tests were made to determine the LD₅₀ dose of fractional local irradiation to the head of hamsters. Ultramicro chemical and bacteriologic tests on the saliva of previously irradiated hamsters continued. Microscopic examinations of all tissues of animals dying during these tests are being made in order to determine changes that could account for the high mortality rate. A new group of hamsters (35 animals) was started on the actual experiment after establishing the normal values of calcium, phosphorus, ammonia nitrogen, total nitrogen, pH, and amylase. Weekly lactobacilli counts were made. After terminating irradiation, the animals will be observed for 2 months. Ten animals were started on a caries-producing diet to study the gross and histologic appearance of hamster caries. Some jaws of irradiated hamsters have been embedded in acrylic, without decalcification for ground sectioning./ The Action of Various Ions upon the Organic and Inorganic Components of Enamel and Dentine. During the past quarter, investigations have concerned the decalcification rate of dentine and enamel in the presence of carefully controlled amounts of anions and cations. It is necessary to complete these tests before proceeding with the action of ions which prevent decalcification of the tooth tissues./ Ecologic Studies of the Oral Microbial Flora during this quarter: factors that control the oral microbial flora, with special reference to bacteria associated with the carious process; the part that salivary mucin plays in the metabolism of the lactobacilli. Studies are also being made to determine whether the organic matrices of enamel and dentine can serve as a source of nitrogen for lactobacilli.

3. Reports. "The Effects of Certain Chemical Compounds upon Dentin," by Burnett, G.W. and Cartel, J. S., J. Dent. Res. 30:477, August 1951

"Experimental Increase in Rate of Eruption and Growth of Rat Incisor by Eliminating Attrition," by Schour, I. and Medak, H., J. Dent. Res. 30:521, August 1951

"Factors in Endodontia Therapy," Cartel, J. S. Accepted by Penn. Dent. J. November 1951

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Dr. Avery. Development and Growth of the Human Tooth. The 1st 3-month period was devoted to collection and preparation of histologic material. Specimens gained have been fixed, embedded, and some sectioned, mounted, and stained./ Many of the human embryos have been weighed, measured, and fixed. A large number of pig embryos acquired from a slaughter-house have been measured and weighed, fixed, and embedded and many already have been sectioned. A series of rat embryos have already been sectioned and preparation of fish, amphibian, and reptilian material is in progress./ All embryo jaws are being serially sectioned and reconstruction of the developing teeth made. As complete a collection of each group as possible is being prepared. Any embryo showing evidence of dehydration, autolysis, or damaged in any way is being discarded. All weight and length averages are correlated with the most recent reliable sources to gain ages of material./ It is hoped that this project will establish an accurate base line of normal development of the human tooth which can be utilized in determining deviations from the normal pattern. The comparative study of less complex development of lower animals will probably reveal other answers related to the human tooth.

Dr. Boyd. Traumatic and Chemical Irritants on Teeth. This first report since initiation of the project 1 April states that cavities have been prepared with the bur and airbrasive technique in 5 human teeth and in 15 dogs' teeth. Histologic sections have been made and for some sections the effect of the operative procedures on the tooth has been evaluated. The cavities prepared were either shallow or so deep that pulp exposure occurred./ In shallow cavities enough dentin was left between the cavity floor and the pulp so that no histologic evidence of injury was observed within the pulp, regardless of the filling material used. These materials have been used: amalgam, silicate cement, self-curing acrylic resin, and crown and bridge cement. In most cases enough irritation occurred to stimulate the formation of an extra thickness of secondary dentin in the areas of the pulp chamber wall subtended by the cavity preparation./ All pulp exposures were immediately capped with $\text{Ca}(\text{OH})_2$. No inflammation of the pulp was visible in these cases, whether exposure had been made with a steel bur or airbrasive material. Where the pulp was exposed with airbrasive material, particles of the aluminum oxides were visible in the pulp tissues. There was no visible reaction on the part of the pulp to these particles./ More cavity preparations are to be made in dogs' and humans' teeth. An attempt will be made to form deeper cavities just short of pulp exposures to determine whether or not the pulp will show changes as the result of severe injury and use of filling materials.

Dr. Day. The Essentiality of Fluorine in Nutrition. Work fell into 3 categories: 1) continuation of observations on the reproductive success of rats on the extremely fluorine-deficient diet as compared with controls given the same diet and a small amount of sodium fluoride; 2) continuation of studies on the growth rate of rats born from mothers on the extremely fluorine-deficient diet. (These animals continue to grow less rapidly than litter mates given the same diet plus a fluoride supplement.) 3) Special attention has been devoted to the preparation of experimental dietary components exceedingly low in fluorine. The reserve was augmented. f. Future Investigations will continue on the relationship between fluorine in bones and the resistance of the bones to fracture.

Dr. M. B. Engel. Changes in the ground substance of the oral tissues in physiologic and pathologic states. (30 April - 31 July 1951) The effect of hormones on connective tissues is being investigated electrochemically in collaboration with Drs. Joseph and Catchpole. Estrogenic and gonadotrophic hormones are being used in a study on the sex skin of the monkey. These fundamental studies should form a basis for interpreting changes in gingival tissues under endocrine influence. This work appears to have important implications in electrolyte metabolism./ Quantitative determinations of the water-soluble mucoproteins of the gingival connective tissue in conjunction with histochemical studies are continuing. Completion de-

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depends upon gathering statistically adequate numbers of specimens./ As to the effects of cortisone on bone, early results indicate increased bone density in the primary spongiosa, reflecting either increased synthesis of ground substance or diminished lysis of bone, or both. Osteocytes and osteoblasts reflect the characteristic gluconeogenesis due to cortisone. The blood mucroprotein levels in these animals are elevated. Integrated interpretation of these findings awaits further work./ Dr. Akamine's studies on cartilage transplants are nearing completion.

Quarterly report rendered 31 October: A paper entitled "Electrochemical Assay of Relaxin" is being prepared in collaboration with Drs. Joseph and Catchpole. It has now been shown that a linear relation exists between the log dose of relaxin and the sodium dilution potential, i. e. the degree of depolymerization of the ground substance is proportional to the logarithm of the dose of the hormone. This relation proves useful in developing a new and objective method of assaying the hormone and may have merit in the assay of other hormones acting on connective tissue./ Determination of soluble mucoproteins in normal and abnormal gingival tissue will soon be concluded./ Effect of Cortisone on Bone. Short-term experiment (5 Mg/day for 3 days) indicates a stimulating effect with increased intracellular glycoprotein (bone precursor?) and glycogen in the osteoblasts and osteocytes. Longer-term experiments (beyond 10 days) show a reversal of this effect with inhibition of bone formation./ Effects of some hormones on the electrochemical state of skin. Altered electrochemical states characterized by a change in cation selectivity, especially with reference to sodium and potassium are being produced in monkey skin with sex hormones and in rat skin with cortisone. It is planned to screen a number of steroid substances, using various skins as test tissues./ Studies on urinary mucoprotein. Since the recently demonstrated urinary mucoprotein may reflect the connective tissue state, excretion of this mucoprotein is being studied in a number of conditions where connective tissue is undergoing change, as following parathyroid hormone injection and in scurvy.

It is planned to complete the study of water-soluble mucoprotein in normal and inflamed gingival tissues. It has been indicated that inflamed tissues contain appreciably greater quantities of water-soluble mucoprotein than healthy tissues./ Further investigations on the physiochemical properties of connective tissue are planned, including diffusion potentials in the sex skin of the monkey and in other skin sites in other species. Preliminary data show these potentials are modified by estrogen and gonadotropins./ Other studies will be: the influence of steroid hormones such as cortisone and compound F, using this approach. Supplementary data will be provided by histochemical studies of the ground substance. Early results provide support for the concept of the selective interaction between ground substance and certain electrolytes, notably Na and K. These tests are summarized in "Electrochemical state of Symphyseal Connective Tissue under Hormonal Influence": Liquid junction potentials were determined in the pubic symphyses of female guinea pigs in several categories: normal; castrate; castrate, estrogen-primed; same, with addition of relaxin; post-relaxin; pregnancy; and postparturition. The 1st 3 groups showed in general tight, unrelaxed symphyses, which gave NaCl dilution potentials indicative of the presence of high densities of immobile, negatively charged aggregates. The last 4 groups showed greater or less degrees of relaxation and dilution potentials for these symphyses indicated lower densities of immobile charges. In highly relaxed states, dilution potentials approached limiting values calculated for aqueous salt junctions./ Similar effects were found for substitution potentials, using potassium, lithium, calcium, and magnesium ions. In relaxed symphyses the mobilities of these ions approached aqueous values. In tight symphyses, selective effects, related to the presence of fixed charges, were noted. Potassium

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in relaxed apophyses.

in particular showed a marked decrease of mobility. The mobility of any cation was found to depend on its aqueous mobility, on the electrochemical state of the tissue, and on a constant characteristic of the ion. From other evidence, hormonal action on apophyseal connective tissue leads to depolymerization of ground substance components. Occasional changes in colloidal charge strictly determined with ionic mobilities and water content of the tissue.

The effects of irradiation with a ray emitter, radioactive P^{32} (chromic phosphate), on epithelial and connective tissue structures of the tongue, using an electrical or spectrophotoelectric method is planned to study connective tissue ground substance and nucleoproteins. From our indication that the beta rays depolymerize the ultrastructural units of the nucleus and of the glycoprotein ground substance.

J. reports: "Bone changes in the ground substance associated with the eruption of the teeth," J. Dent. Res. 30: 372, 1951

"Collagenase-like activity in a salivary fraction" with J. L. Crosby, J. Dent. Res. 30: 453, 1951

"Calculation of serum mucoprotein by parathyroid extract," Res. Proc. 10: 1, 39, 1951 (Abstract)

"Utilization of serum mucoprotein by parathyroid extract", submitted to Endocrinology.

"Hormonal Gingivitis". Presented before Workshop on periodontal disease, University of Michigan, 1951. To be published.

Dr. Kellman. This project on the etiology of periodontal disease followed 2 lines: reparative ability of the animal, and age. It was observed that when local etiologic factors were present the young, due to tissue physiology in this age group, will repair more quickly than the late adult. This is confirmed by clinical observation that marginal periodontitis increases in severity with age. But when a systemic factor is involved, greater changes will be seen in the young individual because of a very active metabolic rate. This confirmed by the clinical observation that periodontitis, a disease of variable metabolic origin, is seen in young individuals. Research to date strongly confirms this hypothesis. However, some of the experiments still have to be finished. The timing of extract intervention is to be followed through. In young dogs with kidney disease a disease of the attachment apparatus is seen. There is a distinct loss of bone around the crowning of the periodontal membrane. No pockets are found in the early stages, only in very late manifestations. These animals were photographed, x-rayed of the jaws taken, and to confirm the above statement a pocket function, guinea parache points were placed between the gingiva and tooth and radiographs taken.

Dr. Kerr. Inhalation Hazards of Air Abrasives. A method for exposing animals to a sizable quantity of air abrasive powder has been revised. Animals can now be kept in a constant atmosphere of abrasive material for any desired period. Eight rabbits are being dusted 6 hours per day and have now received 201 hours of exposure. A 2d group of 9 rabbits are on a similar program. Six guinea pigs were exposed to dust 6 hours per day up to 90 hours at which time all died. A 3d group have been started with exposure reduced to 1 hour per day. Some animals were sacrificed after 13 days to determine the quantity of dust entering the lungs. One group of animals in which dust was forced into the trachea 6 months previously have been sacrificed and the material exposed for examination. The experiments are pilot in type and have proved our methods satisfactory.

The first report on Dr. Marshall-Jay's Epidemiologic Study of Periodontal Disease will be rendered at the end of December.

Abstracts are given of Reports 1139, 1137, 1167, and 1176, prepared by National Bureau of Standards in cooperation with the Federal Research Board.

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"Effectiveness of Vacuum Investing in the Elimination of Surface Defects in Gold Castings," by L. A. H. A., Dickson, George, and Schoonover, I. C. Recently vacuum investors designed to eliminate nodules from the surfaces of dental gold castings by spatulating the investment and investing the wax pattern under a vacuum have been made available to the dental profession. A comparison of a large number of castings made using these investors and castings made by the usual methods showed that the use of the vacuum investing techniques greatly reduced the number of surface defects in the castings.

"Bonding of Plastic Teeth to Heat-Cured Denture Base Resins," by Schoonover, I. C., Fischer, T. E., Gerio, A. F., and Sweeney, J. T. In order to determine whether traces of foreign materials remaining on the surfaces of the teeth might explain the failure of some teeth to bond to the denture base resin a series of experiments was conducted on the bonding strength of teeth which had been waxed up and then cleaned, and teeth which had been treated with tinfoil substitutes. The results showed that traces of wax or tinfoil substitute were sufficient to prevent satisfactory chemical bonding and that the methods of wax elimination commonly employed in dental practice did not effectively remove these materials. Flushing the mold with a 10 percent solution of modern synthetic household detergents was found to be an effective and practical means of removing all traces of the wax, but it did not remove the tinfoil substitute.

"Dental Amalgam: The effect of mechanical condensation upon some physical properties," by Ayge, Summar, Dickson, George, Smith, D. L., and Schoonover, I. C. The effect of different techniques of condensation on the compressive strength, dimensional changes, mercury content and microstructure of dental amalgam was investigated. Two of the three mechanical condensation techniques used produced amalgam specimens which increased in compressive strength more rapidly and attained a higher final strength than did specimens made by the three hand condensation techniques employed. These mechanical techniques also produced amalgam specimens that either shrank or expanded less than did those condensed by the hand techniques. The variation in mercury content of specimens made by different operators using the same technique was about as great as the variation produced by the use of different techniques. No relationship was seen between mercury content and compressive strength, dimensional changes or microstructure.

"Fluid Exchange at the Margins of Restorations due Primarily to Thermal Change," by Nelson, R. J., Holcott, R. D., and Paffenbarger, G. C. An investigation of the thermal dimensional stability of acrylic resin restorative materials revealed that the temperature changes normally occurring in the mouth cause an exchange of fluid at the margins of recently placed restorations in extracted teeth. The volume of fluid exchanged is approximately one percent of the volume of the filling. Restorations made of acrylic resin, amalgam, silicate, gold foil, gutta percha, zinc phosphate cement, and cast gold inlays all demonstrated a marginal permeation of fluid with changes in temperature. This exchange is due primarily to the differential thermal expansion of tooth structure and filling materials and to the thermal expansion of the fluid film which occupies the space between the filling and the tooth occasioned by the setting shrinkage of the filling.

Dr. Schour. On Traumatic and Chemical Variants on Teeth. Now being examined are histologic sections of sound teeth of human patients and of dogs in which cavities have been prepared and filled with zinc oxide and eugenol, calcium hydroxide, gutta percha, silicate cement, acrylic resins, oxyphosphate cement, and gold foil and amalgam. About half of the cavities were prepared with burs, the other half with the abrasive technique. The interval between insertion of the fillings and sacrifice of the animal or extraction of the teeth in human

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patients will range from 1 day to 8 months. Some experiments are still in progress./ Also being examined are histologic changes in the dentin and pulp or molars in white rats after preparation of cavities of varying depths, including pulp exposures and the insertion of 5 % and 2.5% paraformaldehyde./ Under way is development of standard methods of cavity preparation in the teeth of rats as a common base line for subsequent comparisons of the effects of different chemical or physical stimuli and different periods of survival.

Dr. Weinman. Cytologic studies of the oral epithelium in regeneration and in benign and malignant tumors." The main purpose is to find histochemical and cytologic methods by which benign growths can be distinguished from malignant growths. G. Mori published his preliminary observations that large amounts of the enzyme, phosphamidase, can be demonstrated in malignant tumors of different kinds, whereas the enzyme is absent from the normal tissues of origin of these tumors and from benign tumors.

The problem is attacked by: 1) Standardization of the method for demonstrating phosphamidase; 2) application of the method to a study of the distribution of phosphamidase in implanted tumors; 3) in experimentally produced tumors; and 4) in spontaneous oral tumors. During this quarter: 1) Standardization of G. Mori's method for phosphamidase determination had been completed. 2) The phosphamidase content of transplanted tumors in mice has been described. It has further been shown that arrest of growth of transplanted tumors by guanazolo (8-azaguanine) does not affect the phosphamidase content. "The effect of Prolonged Administration of 8-Azaguanine on the Growth of Adenocarcinoma E 0771" has been accepted for publication. 3) The study of mitotic activity in the oral mucosa of the rabbit has been completed. 4) A microscopic study of 25 specimens of human oral lesions to which the above method has been applied. is under way. Collection and preparation of further specimens is in progress. f. Future plan is to concentrate on the differential diagnosis of human oral lesions by the method of phosphamidase demonstration. The method will be applied to a large body of material, specifically to leukoplakia and related lesions./ Tumors of the previously studied area of the oral mucosa in the rabbit will be produced by carcinogens. The mitotic activity and amount of phosphamidase will be studied in different intervals of carcinogenesis.