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THE UNIVERSITY OF CALIFORNIA
Los Angeles Campus
SCHOOL OF MEDICINE
ATOMIC ENERGY PROJECT
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QUARTERLY PROGRESS REPORT
FOR PERIOD ENDING MARCH 31, 1952

Submitted by: Stafford L. Warren, M.D.
Director

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TABLE OF CONTENTS

ABSTRACT

The fifteenth quarterly report being submitted for Contract No. AT-04-1-GEN-12 is issued in accordance with Service Request Number 1 except for the report of the Alamogordo Section, Code 91810, which is submitted in accordance with the provisions of Service Request Number 2.

Work is in progress on continuing existing projects. In addition, new projects have been initiated including the Kinetics and Mechanism of Protein Denaturation (10018); The Effect of Irradiation on the Constituents of Embryonic Serum (30033); and The Use of Controlled Atmospheres for Spectrographic Excitation Sources (40053).

Many of the Project units are either wholly or partially completed and the following initial reports are available: Identification of Ferritin in Blood of Dogs Subjected to Radiation from an Atomic Detonation (UCLA-180); The Nutritional Value of Intravenous Tapioca Dextrin in Normal and Irradiated Rabbits (UCLA-181); The Decarboxylation and Reconstitution of Linoleic Acid (UCLA-183); Preparation and Properties of Thymus Nucleic Acid (UCLA-184); The Radiation Chemistry of Cysteine Solutions Part II. (a) The Action of Sulfite on the Irradiated Solutions. (b) The Effect on Cystine (UCLA-185); A Revolving Specimen Stage for the Electron Microscope (UCLA-178); An Automatic Geiger-Mueller Tube Tester (UCLA-186); The Value of Gamma Radiation Dosimetry in Atomic Warfare Including a Discussion of Practical Dosage Ranges (UCLA-187); and A New Plastic Tape Film Badge Holder (UCLA-189). Two additional reports were issued; one by Dr. Wilbur Selle entitled Attempts to Alter the Response to Ionizing Radiations from the School of Medicine, UCLA (UCLA-176), and two, a restricted distribution report from the Alamogordo Section entitled Field Observations and Preliminary Field Data Obtained by the UCLA Survey Group on Operation Jangle, November 1951 (UCLA-182).

ADMINISTRATIVE DIVISION - Code 90000

Robert J. Buettner
Project Manager

HEALTH SECTION - Code 90780

Pirchard M. Brundage, M.D.
Chief of Section
Helen B. Hale, R.N.

The following Health Department statistics are available for quarter ending February 29, 1952.

I. 842 treatments for minor illness (initial and revisit) and injuries (revisits) were done.

II. 82 minor industrial injuries have been treated.

III. 4 new physicals and 6 termination physicals were done.

PATHOLOGY SECTION - Code 90781

Alvin E. Lewis, M.D.
Chief of Section

Project #00005 - Radiation Effects on Hepatic Function and Correlation with Associated Structural Changes

Alvin E. Lewis, M.D., Raymond D. Goodman, M.D., Jacques Engelhardt, M.D., Dorothy N. Highby, Edward A. Schuck

Work on this project has been temporarily discontinued.

Project #00010 - The Effect of X-Rays on Vascular Permeability to Evans Blue

Alvin E. Lewis, M.D., Raymond D. Goodman, M.D., Jacques Engelhardt, M.D., Edward A. Schuck

During the past quarter studies have been carried out on the kinetics of dilution in the pulmonary vascular bed. This information will be needed in order to estimate the *in vivo* blood volume of the lungs from time-plasma concentration curves obtained following the injection of suitable dyes or radioisotopic indicators. To accomplish this, four animals have been studied by perfusion of the lungs with Evans Blue in saline. Constant flow rates were maintained with Mariotte systems. The time-plasma concentration relationships in the outflow were obtained by passing the fluid through a tube leading to a photomultiplier and recorder system. Results obtained in this manner suggest that dilution in the pulmonary vascular bed is incomplete and that the volume calculated from time-plasma concentration curves will be considerably less than those obtained by post-mortem extraction of dye and hematin by the method previously reported (Report UCLA-117). It is possible, however, that these results are distorted by artifacts of the experimental situation. In this experiment perfusion is

begun 8 to 10 minutes post-mortem. The flow rates maintained by the Mariotte system are lower than the ordinary cardiac output and the perfusion fluid is devoid of protein. In order to overcome these defects, studies are now in progress in which anesthetized animals are used. Constant injections of dye are made into the left auricle and into the base of the pulmonary artery. Records are made on blood obtained by cannulation of the abdominal aorta. The sample tube is led to a recording photomultiplier system as indicated above and the blood returned to the abdominal aorta distal to the first cannulation. Because of the technical difficulties of this experiment, insufficient data have been obtained up to this time to form any definite conclusions regarding the *in vivo* behavior of the pulmonary vascular bed as a diluting system.

Project #00014 - Quantitative Analysis of Gastrointestinal Transit

Raymond D. Goodman, M.D., Alvin E. Lewis, M.D., James Mead, Ph.D. (Biochemistry Division), Dorothy L. Fillerup (Biochemistry Division), Edward A. Schuck

The kinetics of β-Eleostearic acid absorption, blood transport, and disappearance have been studied in rats. Preliminary data revealed very low blood levels at hourly intervals up to 6 hours after oral tube feeding of large dosages of the β-Eleostearic acid. Serial sacrifice experiments, in combination with Evans Blue dye used as the gastrointestinal transit indicator, showed no deficiency in apparent availability for absorption. Experiments in which surgical ligation of the terminal ileum and cecum were performed and followed both by oral and intravenous injections of β-Eleostearic acid, showed definite evidence of excretion of this material into the lumen of both the terminal ileum and colon. This confirms previous notions about excretion pathways for some lipids.

Subsequently, the rate of blood clearance was measured by obtaining multiple blood samples after the intravenous injection of an emulsion of β-Eleostearic acid. In each instance the blood disappearance was exponential and at a high rate. One minute after intravenous injection only 13 to 16% of the initial quantity could be recovered from the blood. At the end of 6 minutes only 7 to 20% was found. In an attempt to localize possible sites of deposition, the viscera and body fat of two rats were completely analyzed for β-Eleostearic acid 8 minutes after an intravenous injection of 30 mgm. From the initial quantity given, one-tenth was found in the liver and much smaller amounts found in the remaining tissues. These data suggest that metabolic conversion rather than simple excretion accounts for the high disappearance rates of β-Eleostearic acid, whether given orally or intravenously. Further investigations using radioisotopic β-Eleostearic acid are now in progress to explore the nature of this metabolic conversion.

HEMATOLOGY SECTION - Code 90784

William N. Valentine, M.D.
Chief of Section

Project #00001 - Studies on Blood Histamine

William N. Valentine, M.D., Harold Mills, M.D., Phyllis Talmadge

This project has been continued in the past quarter. A large number of cases has been studied including a group of patients attending the clinic at Donner Laboratory in Berkeley. These have been particularly valuable for this study because of the long follow-up and the collateral information available such as Fe^{59} studies. Data have been gathered on leukocyte histamine in various clinical conditions with particular emphasis on certain of the poorly understood myeloproliferative syndromes such as polycythemia vera, secondary polycythemia and myeloid metaplasia. These data, together with the phosphatase findings in Project #00011, indicate a striking chemical pattern which seems clearly to distinguish primary from secondary polycythemia and the leukocytosis of polycythemia and myeloid metaplasia from myelocytic leukemia. The histamine of polycythemia vera and myeloid metaplasia is moderately elevated but does not reach the level seen in myelocytic leukemia. The histamine in secondary polycythemia has thus far been normal in every instance. A number of experiments have failed to demonstrate the presence of measurable histaminase activity in normal leukocytes.

Project #00002 - Studies on the Relative Sensitivity of Marrow Erythroid Cells to Roentgen Irradiation

William N. Valentine, M.D., William S. Beck, M.D., Harold Mills, M.D., Leslie Siewert

This project has been partially written up in three reports, Reports UCLA-123, -124, -125, and will appear shortly in scientific periodicals. In brief it was found:

1. After severe hemorrhage, immediately followed by irradiation, recovery from anemia was only slightly slower in the irradiated than in the non-irradiated group subjected to hemorrhage.

2. In contradistinction myelopoiesis was severely depressed for 30-40 days.

3. Ability to regenerate erythrocytes and to restore white cell counts was much the same after four repeated phlebotomies and roentgen irradiations in the same animals. These multiple insults were each separated by several month intervals.

4. The dynamics of erythrocyte regeneration have been analyzed in terms of a series of differential equations.

A series of animals has been studied in which the severe hemorrhage

followed the irradiation. This was intended to rule out the possibility that hemorrhage and surgical anaesthesia prior to irradiation induced anoxia which might be significantly protective. In the group in which surgical hemorrhage followed irradiation, the rates of regeneration of the erythrocytes and leukocytes were essentially similar to those of the earlier group, suggesting that anoxia was not effectively producing radiation protection.

A group of splenectomized animals has been hemorrhaged and irradiated and is now being followed. Data are not yet available in this group. In addition, preparations are being made to study a group of 3 animals with lead-shielded spleens to evaluate the role of the spleen in the rate of marrow regeneration.

Project #00011 - Studies on the Phosphatase Activity of Leukocytes

William N. Valentine, M.D., William S. Beck M.D., Juanita Lamport

As mentioned in Report UCLA-175, this project has been partly completed, reports having been published recently in the Journal of Laboratory and Clinical Medicine (Reports UCLA-115 and -122). Within the past quarter, a large number of data have been collected on cases of polycythemia vera, secondary polycythemia, and myeloid metaplasia, including those of the Donner Laboratory in Berkeley. As mentioned under Project #00001, strikingly different phosphatase patterns are emerging, permitting the differentiation of primary from secondary polycythemia and the leukocytosis of polycythemia and myeloid metaplasia from myelocytic leukemia. The patterns so far established are as follows: (Based on data expressed in terms of milligrams of P liberated from sodium-β-glycerophosphate per 10^{10} cells per hour)

	Alkaline Phosphatase	Acid Phosphatase
Leukocytosis	High	Normal
Chronic myelocytic leukemia	Low	High
Chronic lymphatic leukemia	Low	Low
Acute leukemia	Low	Low
Polycythemia vera	High	High
Secondary polycythemia	Normal	Normal
Myeloid metaplasia	Variable	Normal

Project #00015 - Studies on the Oxidative and Fermentative Metabolism of Leukocytes in Health and Disease

William S. Beck, M.D., William N. Valentine, M.D., Juanita Lamport

On the premise that inquiry into the basic metabolic activities of leukocytes is a necessary preliminary to the understanding of radiation injury and of any manifestation of disturbed leukopoietic physiology, an extensive study has been undertaken on aspects of the oxidative and fermentative

metabolism of leukocytes in health and disease. The program consists of experiments in which leukocytes are isolated from whole blood, homogenized and incubated under various conditions in the Warburg respirometer. In the initial phase of the study, rates of oxygen uptake during the oxidation of glucose are being measured, as well as rates of lactic acid production during aerobic and anaerobic glycolysis. Leukocytes are being obtained from individuals with leukocytosis and leukemia, with normals as controls. It is planned to study the intermediary metabolism of the normal and abnormal leukocytes under various conditions of incubation, clinical status and therapy.

In general it has been found that the normal leukocyte, in a system containing glucose, buffer-pH 7.4, DPN, hexose diphosphate, ATP, and cytochrome C, has a rate of oxygen consumption, glucose consumption, and lactic acid production of a higher order than the leukemic cell. Approximately the same ratio of glucose oxidized to glucose glycolyzed is found in both. Further experiments have confirmed that in mixtures of normal and leukemic homogenates, less oxygen is consumed than the sum of the activity of the individual homogenates. This phenomenon has been subjected to an intensive study: this occurs with pyruvate as the initial substrate but does not appear with succinate as the substrate. Experiments suggest that highest rates of oxygen consumption are obtained only when initial substrates are members of the tricarboxylic acid cycle and that the rate of oxygen consumption under these circumstances is approximately twice that obtained when the initial substrate is an intermediate of the glycolytic cycle. These experiments have been continuing with little over-all change in the reported picture. Further experiments are planned for the next quarter with systematic manipulations of the incubation system.

Data are being expressed in several ways, activity being referred to cell numbers, cell weight and desoxyribose nucleic acid and ribose nucleic acid.

Project #00016 - Phosphorus Fractionation Studies in Leukocytes of Health and Disease

William S. Beck, M.D., William N. Valentine, M.D., Phyllis Talmadge

As mentioned in Report UCLA-175, measurements are being made of leukocyte acid-soluble phosphorus, phospholipid phosphorus, nucleic acid phosphorus, phosphoprotein phosphorus, DNA, and RNA. Extracts of leukocyte material are being digested and measured for phosphorus content.

Considerable data have been accumulated suggesting differences in phosphorus content and partitioning between normal and abnormal leukocytes. DNA and RNA determinations seem to correlate well with morphological

evaluations of the relative size of nucleus and cytoplasm. Data are not yet sufficient to warrant report. These experiments will be continued in the next quarter.

Project #00017 - Studies on Various Aspects of Heme Synthesis in Nucleated Red Blood Cells

Harold Mills, M.D., William N. Valentine, M.D., Richard Riley, Ph.D., Esther Richter

A number of experiments have been carried out in which duck blood (a readily available source of nucleated erythrocytes) has been incubated *in vitro* with N^{15}-labeled glycine to determine the effects of radiation and various inhibitors on the rate of *in vitro* heme synthesis. Measurements of N^{15}-incorporation are made on recrystallized heme by mass spectrographic techniques in the Radiobiology Section. Preliminary studies show, interestingly, that relatively enormous doses of radiation have practically no effect on heme synthesis as it occurs in this system. In addition, nitrogen mustard, in concentrations considerably higher than those which would occur in the therapeutic use of this drug, has no measurable effect. A large number of inhibitors are at present being evaluated. It is hoped that some concept can be worked out regarding the intermediate substances involved in the metabolic pathway of heme synthesis. Toward this end, it is tentatively planned to obtain a number of synthetic N^{15}-labeled postulated intermediates for study in this system.

Hematology Service Unit

Janet Carver, Juanita Lamport, Rodney Valentine

The following service hematology functions have been carried out by the Hematology Section during the past quarter. The tabulated determinations represent initial, terminal, and routine hematologic checks and urinalyses on Project personnel.

(1) PERSONNEL

Erythrocyte counts	72
Hemoglobin determinations	72
Packed cell volume determinations	18
Sedimentation rate determinations	19
Leukocyte counts	75
Differential counts	74
Urinalyses	72

(2) ASSISTANCE TO RESEARCH PROJECTS OF OTHER SECTIONS

Full-time technical assistance (Mr. Rodney Valentine) in the hematologic work of the Radiometabolism Section of the Radiobiology Division (91360).

BIOCHEMISTRY DIVISION - Code 91110

James F. Mead, Ph.D.
Chief of Division

GENERAL METABOLISM SECTION - Code 91120

James F. Mead, Ph.D.
Chief of Section

Project #10007 - Metabolism of the Essential Fatty Acids

James F. Mead, Ph.D., Arthur B. Decker, Dorothy L. Fillerup and Barbara H. Polister

I. A Fundamental Study of the *in vitro* Metabolism of the Unsaturated Fatty Acids by Liver Preparations

In contrast to the results of other workers in the field, it has been found that rat-liver fatty acid dehydrogenase is probably a DPN-activated system. In repeated experiments, dehydrogenation of palmitic acid has taken place in the presence of DPN (and ATP) but not in its absence. Blank oxidation under these conditions has been absent or negligible. It is possible that the activating effect of ATP noted both in these experiments and by other workers may stem in part from its function in protecting the DPN. In any event, the requirements of this enzyme system now appear to have been standardized and other experiments may proceed.

VI. The Metabolism of Linoleic Acid

During the past quarter the main effort in this program has been concerned with the standardization of the techniques used in C^{14} in methodology. These involve the setting up of metabolism cages and the perfection of standard methods of sample preparation and counting. Several trial gross metabolism studies have been run and have indicated that the apparatus is now ready for the extensive use to which it will be put.

In a continuation of the investigation of the effect of x-irradiation on essential metabolites in the presence of unsaturated fatty acids, the study of vitamin A has been concluded.

Table I relates the destruction of vitamin A by irradiation to its mole fraction in admixture with methyl oleate. The emulsions of methyl linoleate and vitamin A were mixed in the desired proportion and irradiated with 1000r at 25r/min in the usual manner.

Table I

Conc. Me Lin. (molar)	Mole % Vit. A.	% Change in Vit. A.
5×10^{-3}	16	-1 ?
1.5×10^{-2}	4	16
2.3×10^{-2}	1.3	28
"	0.26	49
"	0.13	59.

Evidently the destruction of vitamin A depends on its concentration. Some protective effect of vitamin A for methyl linoleate was also noted, since with the higher concentrations of the vitamin, little oxidation of the ester occurred.

The other essential fatty acids have also been studied by irradiation of emulsions of their methyl esters. Qualitatively both methyl linolenate and methyl arachidonate showed increases in diene absorption after 1000r at 25r/min at concentrations of 3×10^{-4} M. The extent of change in each case seemed to be greater with an increase in the number of double bonds, arachidonate showing the greatest effect, but, no quantitative figures can be given until materials of comparable purity can be obtained.

Irradiation of dilute emulsions of two fractions of beef adrenal lipids, both high in arachidonate, showed little alteration in one case and definite oxidation in the other. Evidently, some natural antioxidant was present in one fraction.

These studies will be continued with emphasis in the immediate future on the effect of natural antioxidants and other essential metabolites on the reaction.

Project #10009 - The Effects of X-irradiation Upon Fat Absorption

James F. Mead, Ph.D., Arthur B. Decker, Dorothy L. Fillerup, Leslie R. Bennett, M.D. (Radiobiology Division) and Raymond D. Goodman, M.D. (Pathology Section)

In the study on fat absorption in humans, techniques have been standardized and data on normal subjects is accumulating.

The patient is given a breakfast containing about 30 grams of fat (as milk fat) but no material with pronounced light absorption in the ultraviolet. Five grams of methyl β-eleostearate is included in the meal. A 1 ml sample of blood is then taken as a blank, and further samples are taken at hourly intervals from one to seven hours. These are treated in the usual manner (see previous quarterly reports and Report UCLA-148), and the isooctane solutions are analyzed on the spectrophotometer.

Peak absorption usually occurs at 4-6 hours and tapers off rapidly thereafter in these normal subjects. Interesting phenomena have also been discovered in the spectra of the normal serum lipids and will be reported in detail when they have been given sufficient study.

Project #10016 - The Absorption of Homologous Protein.

No work was accomplished on this project during the last quarter.

Project #10010 - The Effect of Diet on the Production and Growth of Tumors in Mice

Because of the departure of Dr. Norman Nelson, this project is herewith discontinued.

ENZYME CHEMISTRY SECTION - Code 91130

Norman S. Simmons, Ph.D.
Chief of Section

Project #10017 - Isolation and Study of High-Molecular-Weight Naturally-Occurring Polysaccharides and Nucleic Acids

Norman S. Simmons, Ph.D., Stella Chavos and H. Kenneth Orbach

The isolation of pure highly polymerized nucleic acid from calf thymus has been achieved using a method involving isolation at 0° and at a pH near neutrality in approximately one working day. This material has been shown to be electrophoretically and ultracentrifugally homogeneous within the limits of accuracy of these procedures (Report UCLA-184).

Molecular weight determinations are now under way on this material. Sedimentation constants are being determined at the lowest concentration levels commensurate with adequate (1%) accuracy. Diffusion constants are being determined using the Aminco-Stern electrophoresis apparatus which had to be altered slightly in order to maintain the water bath at 20°. Preliminary measurements of the Schlieren patterns obtained on solutions at concentrations of 0.10, 0.75 and 0.050 g/100 ml indicate that a diffusion constant accurate to about 5% can be obtained. In order to avoid boundary disturbances at these low concentrations, it was found that an ionic strength of 0.15 is optimal. Lower ionic strengths are not adequate to diminish particle interactions manifest as a skewed curve.

These measurements will be finished shortly and will be incorporated and discussed in greater detail in a final report.

PHYSICAL BIOCHEMISTRY SECTION - Code 91150

Project #10014 - Streaming Birefringence of Biological Macro-Molecules (New Title: Physical Properties of Biological Macro-Molecules)

A. Sedimentation Behavior of Nucleic Acids

M. D. Schoenberg, Ph.D. and Lee Deutsch

Refinements in ultra-centrifugal techniques which result in increased accuracy and reproducibility in the values obtained for the sedimentation constant have been described in the recent literature. In order to take advantage of these developments, the operations which enter into the calculation of the sedimentation constant have been standardized. With the object of obtaining

good contrast and clarity of the photographic image on Eastman-Kodak 1-D spectroscopic plates, various developing procedures have been tested. Best results are obtained with Eastman-Kodak D-8, developing for 5 minutes at 68°. A micro-comparator is now being used to make measurements on the plates rather than the less accurate method of measuring enlarged tracings which was formerly used. The method Cecil and Ogsten (Biochem. J., 1948) is now being used to compute the sedimentation constant since this treatment has been shown to make full statistical use of the available data.

Investigation of the sedimentation behavior of a new preparation of hyaluronic acid (see Enzyme Chemistry Section, Project #10004) is now under way. The ultra-centrifugal study of changes in lipoprotein and fibrinogen concentration of rabbit plasma before and after irradiation is being continued in cooperation with Dr, R. F. Riley, Radiobiology Division.

Project #10018 - The Kinetics and Mechanism of Protein Denaturation

E. A Carusi and Lee Deutsch

A preliminary experiment involving the determination of the molecular weight of normal bovine serum albumin by means of light scattering measurements has been completed. The purpose of this preliminary run was to assess the accuracy with which the light scattering photometer had been calibrated and to determine whether the protein solutions were sufficiently optically clean to permit accurate scattering measurements.

The proposed method of clarifying the protein solutions consisted of filtering the solvent, which in this case was 0.15 M saline, through Seitz bacteriological filter pads in a series of porosities of 5μ, 2μ and 0.1μ. Filtered solvent was then added to 0.1 gm crystalline bovine serum albumin so that 100 ml of 0.1% solution of protein was obtained. The solution was next centrifuged at approximately 20,000 g for 30 min to remove particulate matter and the supernatant carefully pipetted off by means of a hypodermic syringe. Since high precision was not demanded in this preliminary run, protein concentration was not redetermined after centrifugation. Two-fold serial dilutions of the 0.1% solution to the limit 0.0031% were made by diluting with filtered solvent. Turbidity measurements at each of the six concentrations were obtained for the 5460 and 4570 Å Hg lines.

In terms of light scattering theory, the relationship between the solute molecular weight and turbidity is given by $Hg/\gamma = 1/M + 2\,Bg/RT + \ldots\ldots$ in which H is a constant for the system under investigation equal to

$$\frac{32\pi^3 n_o^2 (dn/dg)^2}{3\lambda^4 N}$$

where n_o is the refractive index of the solvent and dn/dg is the change in

refractive index with concentration. A plot of Hg/γ versus g, where g is the protein concentration, yields a straight line which when extrapolated to zero concentration gives as the ordinate intercept the reciprocal of the solute molecular weight. The molecular weight value thus obtained presented a deviation from the accepted value of sufficient order of magnitude to indicate that better methods were needed to render the solutions optically clear. Work is now in progress to find a more suitable method.

TRACER SYNTHESIS SECTION - Code 91160

David R. Howton, Ph.D.
Chief of Section

Project #10005 - Synthesis and Analysis of Unsaturated Fatty Acids*

David R. Howton, Ph.D., Judd C. Nevenzel, Ph.D., and Gunther Steinberg

Preparation of Tagged Linoleic Acid

David R. Howton, Ph.D. and Judd C. Nevenzel, Ph.D.

A communication to the Editor of the Journal of the American Chemical Society entitled "The Decarboxylation and Reconstitution of Linoleic Acid" (based on material treated in detail in Report UCLA-183) has now been published (J. Am. Chem. Soc., 1952); a discussion of this procedure, designed to implement the preparation of carboxyl-labeled linoleic acid, will be presented at the Buffalo Meeting of the American Chemical Society on March 25.

Meanwhile, work has continued on certain steps in this procedure where it is felt improvements in yield might be expected, on accumulation of starting materials to be used in the projected and imminent synthesis of 1-C^{14}-stearic acid.

Carboxyl-labeled stearic acid, which is to be used by the General Metabolism Section in orienting experiments preliminary to the linoleic acid studies, is to be prepared from cold stearic acid via silver stearate, heptadecyl bromide, the corresponding Grignard, and reaction of the last with C^{14}-enriched CO_2. Since the detailed course of the reaction involved in the preparation of heptadecyl bromide from silver stearate by the action of bromine is of considerable importance with respect to the corresponding step in the linoleic-acid-decarboxylation-reconstitution procedure, this application of the Borodin reaction has received some careful and fruitful study. As exemplified by the present

*In the last quarterly report (Report UCLA-175) it was stated that further work on the problem of The Metabolism of Linoleic Acid (Section VI unddr Project No.10007 of the General Metabolism Section) would be reported under Project No.10005 of This Section. Pertinent metabolism studies will be found outlined in this and future quarterly reports under the original Project No.10007 (General Metabolism Section) heading; those concerned with analytical studies will continue to be reported under the Project No.10005 (Tracer Synthesis Section) heading.

example of silver stearate, the action of bromine on such salts leads to a mixture of three principal types of products:

$$C_{17}H_{35}COOAg \xrightarrow{Br_2} \begin{cases} C_{17}H_{35}Br & I \\ C_{17}H_{35}COOH & II \\ C_{17}H_{35}COOC_{17}H_{35} & III \end{cases}$$

Heptadecyl bromide (I) is the desired product. The regenerated free acid (II), always observed in greater or lesser amounts, is said by Oldham (J. Chem. Soc., 1950) to derive its hydrogen atom from traces of water incompletely removed from the reactants. The so-called "Simonini ester" (III) evidently arises through secondary reaction of the product I with unchanged silver stearate. In past experience with the action of bromine on the silver salts if such acids as palmitic, stearic, di- and tetrabromostearic, free acids (type II) have always been encountered among the products and have been removed, perhaps incompletely and certainly inconveniently because of emulsion formation, by conventional means. Little or nothing has been known about the amounts of ester (e.g., III) in these products except for the possibly related fact that overall material balances have left much to be desired. For example, in the preparation of pentabromoheptadecane (see Report UCLA-183) only about 42% of the starting silver tetrabromostearate was accounted for as desired bromide (type I) and recovered free acid (type II).

In the present study, 100 g stearic acid was converted to the silver salt by adding silver nitrate to a solution of the ammonium salt of stearic acid in 95% ethanol, yield 98.3%, dried to constant weight in vacuo and stored over P_2O_5. Adding a solution of bromine in carbon tetrachloride to a slurry of this silver salt in the same solvent followed by removal of the resulting silver bromide by filtration left a solution of the mixed products of the reaction, aliquots of which were submitted to several procedures with the principal object of separating acidic from neutral materials. Passing samples of the mixture in a variety of solvents through a column of IR410 ion-exchange resin resulted in no significant removal of the acidic material (in contrast to reports of Cason et al., J. Org. Chem., 1950, in a related case). Predicting that I should be more volatile than II because of its lower polarity (the much higher molecular weight of III making it even more non-volatile), a rapid short-path distillation of the mixture at 1 mm resulted (as shown by base titration) in some concentration of neutral material; however, it was apparent that acceptably complete separation would require considerably more efficient fractional distillation -- a technique presenting formidable obstacles in application to such high-boiling substances. Classical extraction of acidic material with aqueous base affected the desired separation, but

emulsion formation complicated clean phase separation and may very probably have led to some retention of neutral materials (among them the desired product I) in the aqueous phase.

In contrast to these questionably successful methods of resolving this mixture, chromatography on alumina appears to be of considerable promise. Thus a carbon tetrachloride solution of about 16.6 g of the product mixture was poured onto an 8 x 21 cm column of about 700 g of 2:1 alumina-celite mixture prewashed with 750 ml of 60-70° petroleum ether. After development with 1500 ml of the same solvent, the column was sucked dry, extruded from the tube and cut into four approximately equal segments. Evaporation of the eluate left 14.2 g of low-melting neutral material (I) (reported m p 32°). Extraction of the column segments with ethanol gave 1.16 g of a second neutral crystalline material distributed fairly evenly over the whole column, but concentrated toward the bottom; this material -- crude m p 55-59°, raised to 62-64° by repeated recrystallization from ethanol -- is apparently the Simonini ester, heptadecyl stearate (III), reported m p 64.5°. Interestingly enough these ethanol extracts of the column contained negligible amounts of acidic material. Extraction of the top quarter of the column with EtOH-HCl gave 1.0 g of the free acid (II); mere traces of I were extractable in the same way from the second quarter. It is thus clear that free fatty acids are held very tenaciously on alumina columns; this finding not only makes chromatography under these conditions the method of choice in separating the products of the Borodin reaction on silver stearate, but also invites its application to related problems and lends strong experimental support to the efficacy of the practice -- common in these laboratories -- of removing traces of free acids from crude unsaturated fatty acid esters by chromatography on alumina. Support for the supposed Simonini ester structure of the 64° neutral product was furnished by an infrared absorption spectrum of the substance which showed, as expected, an intense ester-carbonyl peak at 5.75μ. Speaking well for the clean separation of the two neutral products (I and III) under these conditions, infrared spectra of various samples of the heptadecyl bromide (I) show negligible absorption in this region.

From this chromatographic analysis, it is clear that the silver stearate has given 79.5% bromide I, 6.4% regenerated acid (II) and 7.9% Simonini ester (III) -- products 93.8% accounted for. Under conditions designed more for analytical than for preparative purposes, Oldham (vide supra) has reported 89.4% I, 5.5% II and no III; it seems quite conceivable (and Oldham's data do not exclude this possibility) that his "I" is in fact a mixture of I and III.

Additional studies of the application of these reactions to tetrabromostearic acid have established the following new facts: 1) Preparation of silver tetrabromostearate as described in Report UCLA-183 gives a 79.4% yield of the desired salt in analytical purity (Found: Ag, 15.22%; Calc'd, 15,27%); this is first crop material; attempts to procure additional amounts of the substance (which would seem to be more soluble in absolute methanol than was heretofore believed) are not yet complete. 2) Running the Borodin reaction on this silver salt by addition of bromine to it (the reaction has been run in reverse manner previously) gives a crude product containing a 20% yield (by base titration) of the regenerated free acid; further analysis of this crude product by the chromatographic procedure described in detail above sholld provide much information of immediate interest. 3) Attempts to convert pentabromoheptadecane to 8,11-heptadecadienyl bromide (the precursor of reconstituted linoleic acid) by debromination with zinc in isopropyl ether containing small amounts of concentrated aqueous hydrobromic acid were completely unsuccessful; on the other hand, careful repetition of the previously employed ethanol-benzene procedure of Silberman (see Report UCLA-183) showed that the desired dienyl bromide could be obtained, even on a small scale, in 88.5% yield (previously reported, 59%).

Carbon Dioxide Material Balance in Low-pressure Carbonation of Grignards. In attempts to provide experimental explanation for discrepancies between amounts of barium carbonate converted to carbon dioxide and yields of carboxylic acids based on Grignard used or carbon dioxide consumed, a single run has shown that 93% of the CO_2 generated from $BaCO_3$ in a high-vacuum manifold could be accounted for in the absence of Grignard. In the presence of Grignard, using excess heptadecyl bromide to react with all the magnesium turnings, the best recovery of CO_2 was 88% of the $BaCO_3$ taken. The yield of stearic acid on the basis of $BaCO_3$ was 31-40% in three cases and 63% in a single run of questionable reliability. Future work will use aliquots of a standard Grignard solution in attempts to achieve (1) better total recovery of added CO_2, (2) better yields of acid on the basis of $BaCO_3$ taken, and (3) better yields of acid on the basis of bromide taken.

Studies on the Degradation of Unsaturated Fatty Acids

Gunther Steinberg

Further studies of the conversion of polybromo fatty acids to the corresponding polyacetoxy compounds, using the 115°-tetrabromostearic acid (TBS) from linoleic acid as a model substance, have included a repetition of the most successful previous run. A mixture of 26.8 g silver acetate, 34 ml glacial acetic acid and 8 ml acetic anhydride was heated for 45 min, then

12 g of TBS was added. After maintaining at 120° and stirring for 15 hours, the mixture was diluted with acetic acid, filtered, solids washed with acetic acid and ether, HCl passed into the filtrate to remove silver, and the residual solution freed of solvent. The residue was saponified with 8.5% alcoholic KOH, 15 min at 100° and overnight at room temperature, giving 2.63 g (36%) of crystalline potassium sativate (tetrahydroxystearate) in two crops, one at room temperature, the other at 0°. Neutralization of the residual material with H_2SO_4 followed by removal of solvents and exhaustive extraction of the residue with ether gave 0.71 g of solid and 3.08 g of oily material. The oil was shown to contain some halogen and, by the periodate oxidation procedure discussed below, about 1.4 vicinal glycol units per molecule; an ultraviolet absorption spectrum of this fraction showed maxima at 233, 258, 288, 243, and 316 mμ, the significance of which remains obscure.

Periodate Oxidation of Tetrahydroxystearic (Sativic) Acids

Oxidation of 172-4° (Run 1) and 152° (Run 2) sativic acids (from permanganate hydroxylation of linoleic acid) by paraperiodic acid in 50% aqueous ethanol (pH 1.7-2) has furnished the following results:

Time elapsed (hrs)	H_5IO_6 consumed (mole-equivs.)	
	Run 1	Run 2
0.05	1.09	-
.066	-	1.14
.17	1.36	1.36
.33	1.58	1.63
.50	1.70	1.76
.75	1.78	1.92
1.0	1.87	1.92
1.5	1.96	1.98
2.0	2.04	2.09
2.5	2.02	-
3.0	2.02	2.17

It is clear from these data that the two isomeric sativic acids behave essentially identically, two mole-equivalents of the reagent being consumed in about 100 minutes; oxidation under these conditions is not appreciably more extensive than that involved in fission of the molecule between vicinal glycol carbon atoms. Products of this reaction are presumably n-hexaldehyde, malondialdehyde and azelaic semialdehyde.

In contrast to the straightforward classical glycol cleavage resulting under weakly acidic conditions, oxidation in half-saturated sodium bicarbonate solution (pH 8-8.5) is both more rapid and more extensive; here Run 3 involved the 152° sativic acid used above in Run 2 and Run 4 a 175° sativic acid obtained from 115°-tetrabromostearic acid via reaction with silver acetate and saponification (see above).

Time elapsed (hrs)	H_5IO_6 consumed (mole-equivs.)	
	Run 3	Run 4
0.25	-	2.06
0.4	2.51	-
1.67	4.14	-
2.0	-	4.07
4.0	4.96	-
4.5	-	4.86
6.0	5.22	5.18
7.5	5.38	-
7.8	-	5.43
24.0	-	6.56

Here again the isomerism of the tetrahydroxystearic acids exerts little effect. But in 15 minutes, oxidation is already as extensive as in the runs at pH 2 and further reduction of periodate occurs, presumably in destruction of the sensitive malondialdehyde intermediate (cf Huebner, Ames and Bubl, J. Am, Chem. Soc., 1946). Conversion of this dialdehyde to three moles of formic acid, requiring 5 mole-equivalents of periodate in all, is complete in about 5 hours, further consumption of oxidizing agent supposedly being involved in oxidation of formic acid to carbox dioxide, Although of possibly less utility for analytical purposes because of its less straightforward course, the more extensive oxidation at basic pH's may prove to be advantageous from the standpoint that the central three-carbon group of the 1,4-diene system of unsaturated fatty acids of the "essential" type are thereby converted to more tractable oxidation products.

Synthesis and Characterization of Unsaturated Fatty Acid Oxidation Products

Gunther Steinberg, and Judd C. Nevenzel, Ph.D.

Using the corresponding acetal (1,1,3,3-tetraethoxypropane--kindly supplied by the Carbide and Carbon Chemicals Corporation) as a possible source of malondialdehyde, it was shown that although this substance is immiscible with dilute HCl, a mixture of the two in the course of standing together over a weekend at room temperature becomes a homogeneous brown solution, the homogenization indicating hydrolysis and the development of the brown color indicating more or less extensive alteration of the highly reactive free dialdehyde. A solution of the acetal and 4 mole-equivs. paraperiodic acid in 50% aqueous ethanol gave the following results:

Time (hrs):	2	4	6	23	45
Equivs. H_5IO_6 consumed	0.61	0.72	0.73	0.87	1.07

In view (see Runs 1 and 2, above) of the apparent resistance of malondialdehyde to periodate-oxidation under such conditions, the consumption of oxidizing agent here is of interest.

Some preliminary study of the potentialities of conversion of the periodate-oxidation products of hydroxylated unsaturated fatty acids to 2,4-dinitrophenylhydrazones, followed by paper chromatographic separation, has been made. Oxidation products of a sample of 9l°-dihydroxystearic acid (from oleic acid via performic acid treatment) -- presumably pelargonaldehyde and azelaic semialdehyde -- were subjected to such a procedure. Development of the paper chromatogram using an ascending solvent (90-100° petroleum ether) front gave three spots -- one strong, R_f 0.5, and another, probably representing 2,4-dinitrophenylhydrazine, which did not move under these conditions. Similar treatment of a sample of a sativic acid also gave only two moving spots, whereas three would be expected; this observation indicated that working up of the oxidation mixture must be modified to assure complete extraction of all products of the oxidation.

Some further study, along lines discussed in earlier quarterly reports, has been devoted to attempts to prepare an authentic sample of glutaric acid semialdehyde 2,4-dinitrophenylhydrazone, one of the products anticipated from oxidative degradation of arachidonic acid. An attempt was made to repeat the Linstead-Wang (J. Chem. Soc., 1937) preparation of 2-(2',4'-dinitrobenzeneazo)-2-carbethoxycyclopentanone; the sample obtained had a melting point in good agreement with that reported, but analyzed as containing a molecule of acetic acid. Acid hydrolysis of this substance gave a complex mixture containing, as shown by chromatography on alumina, at least four components.

Preparation of Methyl β-Eleostearate

David R. Howton, Ph.D.

Because of considerable promise of this ester as a means of studying *in vivo* fat absorption and related problems (see report of General Metabolism Section), attempts have been made in the course of preparing a rather large amount of the substance to improve conditions involved in its preparation from tung oil; particular attention has been paid to the rather extraordinary sensitivity of the substance toward oxidation and polymerization. In all manipulations air was excluded as completely as possible (using atmospheres of CO_2 or N_2) and exposure to light from fluorescent lamps was kept at a minimum. Principal improvement over older methods is concerned with the esterification step, previously accomplished by refluxing the recrystallized β-eleostearic acid in 1 N MeOH-HCl for two hours; esterification with 1 N MeOH-H_2SO_4 overnight at room temperature was found to be equally complete and much less destructive of the desired ester, judging from color of the esterification mixture and considerably improved yield of the desired product. Following treatment of

the esterification mixture with ice-water, the crude ester was taken up in petroleum ether, dried and passed through an alumina column. Four successive 500-ml eluates from the column contained 77.3, 29.2, 6.2 and 3.5 g of water-white ester, total yield 77.5% from the recrystallized acid. Separate examination of each of these fractions by ultraviolet spectrophotometry showed them to be essentially identical; this finding indicates that the product is stereochemically homogeneous or, what seems much less likely, that the alumina column is incapable of resolving mixtures of the geometric isomers of this conjugated triene acid Freed of traces of petroleum ether by warming rapidly to 100° (steam) while applying a high vacuum (less than 1 mm), the pooled ester fractions give a product crystallizing readily and completely at refrigerator temperatures.

PHARMACOLOGY AND TOXICOLOGY DIVISION - Code 91210

Thomas J. Haley, Ph.D.
Chief of Division

TOXICOLOGY SECTION - Code 91220

Lawrence E. Detrick, Ph.D.
Chief of Section

Project #20004 - Coagulation of the Blood Before and After X-irradiation

Thomas J. Haley, Ph.D. and Bonnie Rhodes

Since the last quarterly progress report (Report UCLA-175), studies have been made of the effect of Toluidine Blue on the coagulation time of a purified system containing bovine fibrinogen and thrombin using the following procedure: 0.1 cc of varying concentrations of Toluidine Blue was mixed with 0.2 cc of bovine fibrinogen (1% solution in saline) and allowed to stand for 2, 5 or 10 minutes; then, 0.2 cc of bovine thrombin (0.5 units/cc) was added and the clotting time recorded. All determinations were made in triplicate until 12 to 15 had been completed for each concentration of Toluidine Blue (5, 10, 15, 25, 50, 75 and 100 γ/0.1 cc). At all time intervals, doses of 5 and 15γ of Toluidine Blue increased the clotting time. With the 2 minute time interval, 10γ of Toluidine Blue decreased the clotting time slightly but the time was still above the control level. With the 5 and 10 minute intervals an increased clotting time was noted. At all time intervals, 75γ of Toluidine Blue decreased the clotting time an average of 36% from that of the control level. Doses above 75γ produced incoagulability regardless of the time interval. Studies will be conducted with the electrophoresis apparatus to attempt to determine the effect being produced on fibrinogen by Toluidine Blue.

Project #20010 - The Effect of Intravenous Feeding upon Mortality Due to Acute Whole Body Radiation

Lawrence E. Detrick, Ph.D. and Virginia Debley

Histological interpretation of tissue specimens from animals receiving intravenous oxypolygelatin will be made on all sections simultaneously immediately following the conclusion of the final OPG medicated animal group now in progress. The livers of the OPG injected animals just concluded were complicated by considerable gross pathological change, probably coccoidiosis from appearance, but this will wait histological confirmation.

Project #20011 - The Effect of Potentiation and Inhibition of Acetylcholine on the Intestinal Damage Due to Acute Whole Body Radiation

Lawrence E. Detrick, Ph.D. and Virginia Debley

The investigation will continue with reduced dosages of atropine (25 and

100 mg/kg) in radiated medicated and non-radiated medicated and similar saline control groups. Prostigmine (.06 mg/kg) will be given to a second animal group. Radiation administered locally to the abdomen will be increased from 1250 to 1500 r.

Project #20020 - The Pharmacological and Toxicological Effects of Several Phenazine, Oxazine and Thiazine Dyes

Thomas J. Haley, Ph.D. and James L. Leitch, Ph.D.

Since the last quarterly report (Report UCLA-175), studies have been made on the antagonistic action of Toluidine Blue, Azure A, Neutral Red and Neutral Violet against the pilocarpine- and nicotine-induced spasms in the isolated guinea pig ileum. The procedure used in all tests was as follows: After the highest pD dose of drug had been allowed to act on the isolated tissue in Tyrode's solution for two minutes, the standard dose (0.5 ml of pD 4.00) of pilocarpine or of nicotine was added to the tissue bath. After one minute, the tissue was washed twice. Following a five-minute recovery period, the procedure was repeated using the next lower pD value of the drug. All results are based on the average of three ileum samples from three different guinea pigs. All data were analyzed statistically by the Litchfield-Wilcoxon method and are summarized in Table II for pilocarpine and Table III for nicotine.

TABLE II

Antagonistic Action of Four Antiheparin Dyes Against the Pilocarpine-Induced Spasm of the Isolated Guinea Pig Ileum

	ED_{50} Data			Test of Potency(2)		Slope Data			Test of Parallelism(2)	
Drug	ED_{50} γ/ml	Range(1) γ/ml	$f_{ED_{50}}$	PR	f_{PR}	Slope	Range(1)	f_S	SR	f_{SR}
Atropine Sulfate	0.22	0.08-0.59	2.66	---	---	4.47	1.53-13.0	2.92	---	---
Toluidine Blue	0.71	0.11-4.65	6.55	3.22	8.32	5.23	0.93-29.6	5.65	1.93	7.85
Azure A	2.80	0.93-8.40	3.00	12.72	4.34	5.37	2.43-11.9	2.21	1.32	3.80
Neutral Red	6.80	2.84-16.2	2.38	30.9	3.70	2.55	1.30-5.00	1.96	1.49	3.55
Neutral Violet	5.10	2.14-12.1	2.38	23.2	3.70	2.96	1.36-6.46	2.18	1.34	3.76

(1) For 19/20 confidence limits.

(2) Data for atropine sulfate used as reference standard.

TABLE III

Antagonistic Action of Four Antiheparin Dyes against the Nicotine-Induced Spasma of the Isolated Guinea Pig Ileum

Drug	ED_{50} γ/ml	ED_{50} Data Range(1) γ/ml	$f_{ED_{50}}$	Test of Potency(2) PR	f_{PR}	Slope Data Slope	Range(1)	f_S	Test of Parallelism(2) SR	f_{SR}
Atropine Sulfate	1.7	0.40-7.2	4.23	---	---	6.07	1.88-19.6	3.23	---	---
Toluidine Blue	8.7	3.42-22.1	2.54	5.11	5.56	2.74	1.21-6.20	2.26	2.22	4.16
Azure A	5.4	1.05-27.7	5.13	3.17	8.84	15.1	2.40-95.0	6.30	2.49	8.85
Neutral Red	3.4	1.04-11.1	3.28	2.00	6.46	5.26	2.34-11.8	2.25	1.15	4.06
Neutral Violet	10.5	4.3-25.6	2.44	6.17	5.45	2.63	1.29-5.36	2.04	2.31	3.94

(1) For 19/20 confidence limits.

(2) Data for atropine sulfate used as reference standard.

From the data in Tables II and III it is apparent that all lines are essentially parallel. The four antiheparin dyes have approximately the same potency for antagonizing the nicotine-induced spasm. However, for antagonizing the pilocarpine-induced spasm, Toluidine Blue has approximately the same potency as atropine sulfate while Azure A, Neutral Red and Neutral Violet are less potent. In addition it was noted that all tissues became heavily stained and very little of the dye could be removed by repeated washing of the tissue. Furthermore, after such washing, the sensitivity of the ileum to the standard drugs was not regained, indicating an irreversible toxic action of the dyes.

In addition to the studies on the isolated guinea pig ileum, investigations have been started on the effects of Toluidine Blue, Azure A, Neutral Red and Neutral Violet on the heart rate, electrocardiogram, blood pressure and respiration of dogs under Dial-Urethane anesthesia. All four dyes were studied at doses of 0.5, 1.0, 2.5, 5.0, 7.5 and 10.0 mg/kg body weight. A preliminary examination of the results so far obtained indicate that:

(1) All four dyes stimulate respiration.

(3) There is no apparent effect on the heart rate.

(3) Toluidine Blue and Azure A cause an increase in blood pressure but Neutral Red and Neutral Violet reduce the blood pressure.

(4) There is an apparent potentiation of both epinephrine and acetylcholine with no evidence that either of these two drugs are blocked by any of the four dyes.

(5) The above effects do not seem to be prevented by prior atropinization.

Quantitative interpretation of these data must wait until all experiments have been completed.

Project #20025 - Studies of the Mammalian Capillary Bed Under the Influence of a Large Variety of Autonomic Drugs Both Before and After X-irradiation.

Thomas J. Haley, Ph.D., Margaret R. Andem and Peggy Lenney

Since the last quarterly progress report (Report UCLA-175), a report dealing with the appearance and identification of ferritin in the blood of dogs subjected to radiation from an atomic detonation has been declassified. The blood was obtained by venipuncture for two days pre-irradiation and daily up to and including the eighth post-irradiation day. It was found that the vasodepressor material was stable for preolonged periods when the blood samples were kept frozen, but freezing and thawing caused a rapid loss in activity. The complete details of this investigation appear in Report UCLA-180, a summary of which is given below.

Studies on blood plasma samples obtained from eight dogs subjected to a lethal dose of radiation from an atomic detonation demonstrated that a material was present which, when administered intravenously, decreased the rate of vasomotion and decreased the epinephrine sensitivity of the mesoappendix capillary bed of the normal rat. After its appearance in the blood, the concentration of this vasodepressor material decreased as a function of time. This VDM has been shown to fit the criteria established for ferritin.

Studies have been made on the ability of the livers of normal and x-irradiated rats to inactivate crystalline ferritin. Although it is too early in the investigation to definitely state that the ferritin inactivating enzyme system of the liver is damaged by irradiation, the work thus far points in that direction. The normal liver can inactivate at least 2.2γ/ml of ferritin whereas the irradiated liver can only inactivate 0.022γ/ml. Further work will undoubtedly establish a definite pattern which can be correlated with the blood levels of circulating ferritin in the irradiated animal.

Inasmuch as Zweifach (personal communication) stated that the kidney, liver and adrenals of an animal in physiological shock gave an aberrant staining with tetrazolium chloride, we have investigated the staining characteristics of these tissues from x-irradiated animals. The adrenal and liver tissue sections are difficult to interpret but the kidney sections show a definite staining change after irradiation. The control sections deposit the dye in a regular pattern in the walls of the tubules whereas the irradiated sections have large crystalline deposits laid down in a helter skelter manner. This test, while preliminary in nature, adds additional evidence to the hypothesis that irradiation damage and physiological shock states produce similar changes in the animal organism.

Project #20028 - A Study of the Effect of Pre- and Post-Medication with Flavonoid Compounds on Roentgen Ray Irradiation Mortality

Thomas J. Haley, Ph.D. and Samuel Mann

Since the last quarterly progress report (Report UCLA-175), studies on the effect of catechin in modifying the lethality of x-irradiation in guinea pigs has been undertaken. As yet it is too early to detect definite trends with this compound. However, the other similar compounds had effects which may be summarized as follows: Post-irradiation medication with rutin, hesperidin, hesperidin methyl chalcone and naringin increased the rate of mortality and hesperidin methyl chalcone the total mortality. Pre-irradiation medication with hesperidin and naringin also increased the rate of mortality. The other two flavonoids had no effect on the rate of mortality when given prior to irradiation. It is concluded that evidence thus far obtained indicates that the flavonoids are of no benefit and may be highly detrimental to the irradiated animal.

Metabolic Fate of Flavonoid Compounds

Thomas J. Haley, Ph.D. and Murray Bassin

Work has continued on the influence of the flavonoid compounds on ethereal sulfate, glucuronide and total phenol excretion in the rat after subcutaneous injection of a single dose of 100 mg. Difficulties have been encountered in the total phenol determination and results obtained will be reported at a later date. Results obtained with the other procedures are summarized in Tables IV and V.

Table IV

Hesperidin Methyl Chalcone Excretion - First Day

Rat	Per Cent Increase in Ethereal Sulfate	Per Cent Increase in Glucuronic Acid	Equivalent of mgs of Hesperidin Methyl Chalcone
2364♀	-1.0	4.07	0.478
2361♀	-6.01	39.3	5.22
70♀	-0.6	35.5	6.54

Table V

Rutin Excretion

First Day Sample after Injection

Rat	Per Cent Increase in Ethereal Sulfate	Per Cent Increase in Glucuronic Acid	Equivalent of mgs of Rutin
2306	decrease 3%	27.7	11.68
2332	increase 1.7%	29.4	5.03
2275	decrease 2.8%	13.9	6.63

Only data on the first day's excretion is given because these compounds are apparently entirely metabolized and excreted within the first 24 hours.

Project #20030 - Screening Pharmacological Agents for their Effect on Radiation Damage

Thomas J. Haley, Ph.D., Bonnie Rhodes and Samuel Mann

In 1949, Larkin (Am. J. Roentgenol. Rad. Therap. 62, 547:1949) reported that atropine delayed the mortality in x-irradiated mice. We have started a screening procedure to evaluate atropine and related cholinergic blocking agents for their ability to decrease the rate of mortality and perhaps the total mortality under the same conditions and drugs dosages used by Larkin. The mice, weighing an average of 20 gm, were subjected to 550 r acute whole body x-irradiation with two 250 KVP Picker Industrial Units. The radiation factors were: 250 KVP, 15 MA, FOD 100 cm, filters 0.21 mm Cu inherent, 0.5 mm Cu parabolic and 1.0 mm Al, rate 18r/minute. All animals were irradiated at one time. The mice were injected intramuscularly daily using alternate thighs, beginning 24 hours prior to irradiation and continuing until all but 5 per cent of the animals were dead. The volume injected was kept constant at 0.1 cc and the dosage levels were 0.45×10^{-3}, 0.90×10^{-3} and 1.35×10^{-3} molar. The control group received 0.9 per cent saline. The results obtained thus far are given in Table VI. The statistical analysis is by the method Litchfield.

Table VI

Effect of Cholinergic Blocking Agents on Radiation Mortality

Drug	Group	ST_{50}	Range	Slope	Range
Saline	0.9%				
Atropine	0.45×10^{-3}				
	0.90×10^{-3}	9.1	8.4 to 9.9	1.21	1.14 to 1.29
	1.35×10^{-3}				
Saline	0.9%	7.0	6.4 to 7.7	1.245	1.16 to 1.33
MK-02	0.45×10^{-3}				
	0.90×10^{-3}	10.7	9.83 to 11.62	1.216	1.14 to 1.29
	1.35×10^{-3}				

There were no differences in total mortality with either drug and no difference in the rate of mortality with the atropine medicated groups. However, MK-02 (tropine benzhydryl ether methane sulfonate) in all doses decreased the rate of mortality significantly ($P = 0.5$). Other drugs of these series are being investigated but it is too early in the experiment to draw definite conclusions.

LUNG TRANSPORT SECTION - Code 91230

George V. Taplin, M.D.
Chief of Section

Project #20006 - The Effect of Particle Size on Pulmonary Absorption and Distribution

George V. Taplin, M.D., James S. Grevior, M.D., Camille Finnegan and Philip Noyes

A technique for measuring the lymphatic absorption of colloidal sized particles has been devised. It consists of instilling a measured volume of colloidal pontamine sky blue dye into the trachea through a hypodermic needle, followed by serial sacrifice studies and microscopic examination. It has been shown that this dye is rapidly engulfed by the lung phagocytes and within 4 hours approximately 25 percent is intracellular. By 24 hours nearly all particles have become phagocytized. After intervals exceeding 48 hours, there is evidence of progressive clearance of the dye from the lung macrophages. At one week, only 10-20 percent of the phagocytes still contain dye particles. From these preliminary observations in 15 rabbits, it became apparent that if one could extract the dye from tissue specimens, one could determine quantitatively the amount of dye absorbed from the lungs by way of the macrophage-lymphatic system. Furthermore, it was found that a 1:1 mixture of colloidal prodigiosin and pontamine sky blue is stable. The former is chloroform soluble and the latter remains in the aqueous phase. By using this dye mixture, one can also determine whether different types of colloidal particles behave in similar or different fashion in respect to phagocytosis.

In preparation for applying this technique in a large series of normal and irradiated animals, the peak absorption band of pontamine has been determined (600 mμ) and a standard curve showing the relation between concentrations of the dye and percent transmission has been prepared (See Fig. 1).

At present, the extraction of this dye from lung tissue is presenting a problem because of the apparent affinity of the dye for protein material.

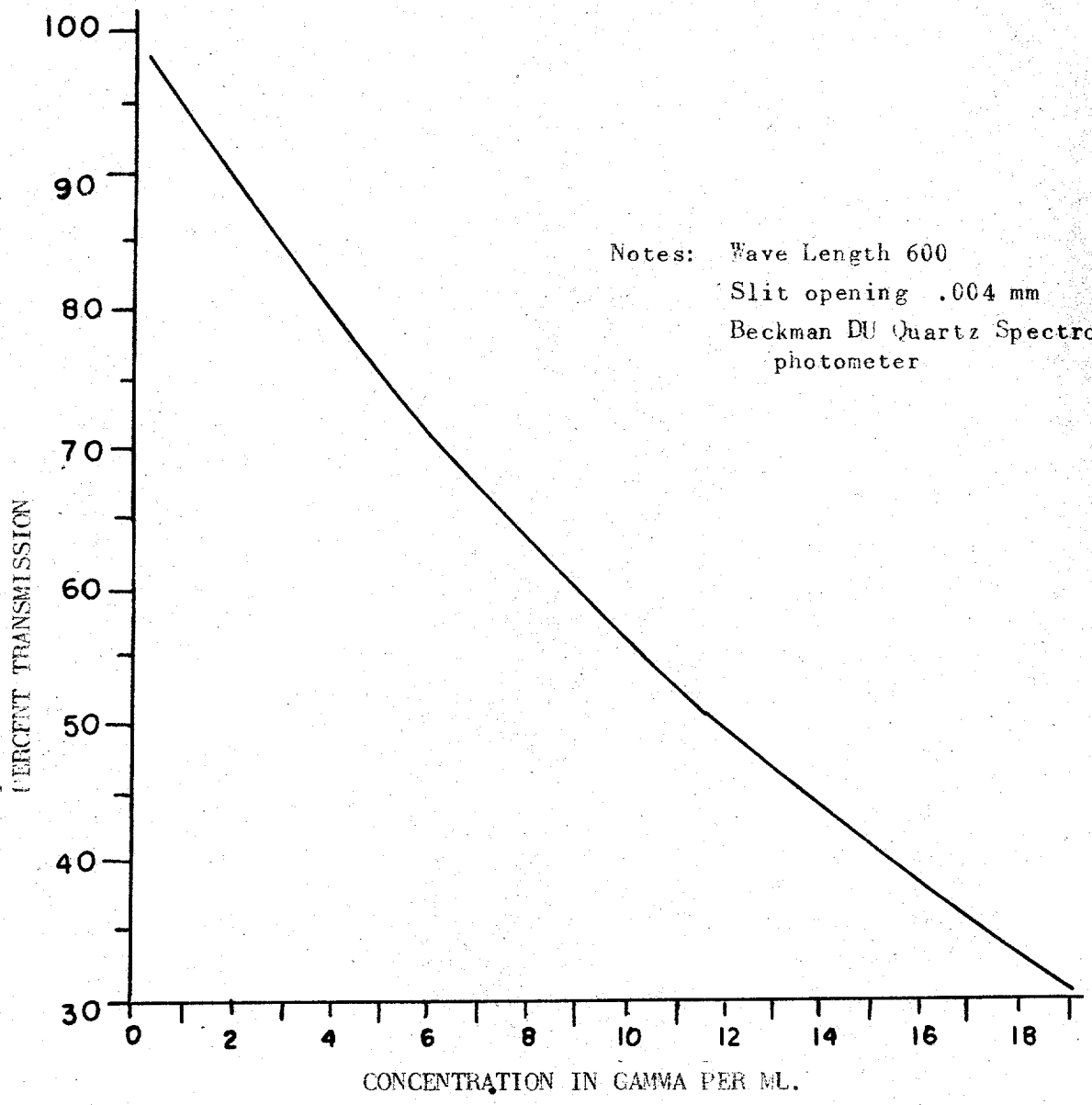
Project #20007 - The Effect of Cilia upon Pulmonary Absorption and Distribution

George V. Taplin, M.D., James S. Grevior, M.D., Camille Finnegan and Philip Noyes

The intravenous studies with colloidal prodigiosin (Report UCLA-175) and its distribution in the body of normal and x-irradiated rabbits during the first 30 days post-irradiation have been completed. A detailed account of these experiments, correlated with similar lung clearance investigations, is being made the subject of a separate UCLA report.

Fig. I

PONTAMINE SKY BLUE CONCENTRATION
vs. PERCENT TRANSMISSION



Notes: Wave Length 600
Slit opening .004 mm
Beckman DU Quartz Spectrophotometer

Project #20012 - Dust Distribution and Absorption from the Pulmonary Tree

George V. Taplin, M.D., James S. Grevior, Camille Finnegan and Philip Noyes

A problem related to pulmonary absorption from the lymphatic system has been given some attention. The anemia of some animals surviving large doses of radiation has been shown to have many characteristics suggestive of hypersplenism. Two methods have been used to assess the function of the macrophage system in these animals. One has been to make determinations of the direct Coombs reaction, the other to measure the rate of prodigiosin blood clearance. The Coombs reaction has failed to show evidence of abnormal antibody on the surfaces of either normal or irradiated anemic rats' red cells.

The results of prodigiosin blood clearance studies on radiated vs normal rabbits are presented in Table VII.

The studies with doubly tagged bacteria, reported in Report UCLA-175 have been temporarily suspended.

Table VII
Comparison of Prodigiosin Concentration in the Plasma of Normal vs Radiated Rabbits

	Normal Rabbits	Radiated Rabbits
Number of Animals	28	7
Prodigiosin Concentration µg/ml at 5 min after i.v. mean values	0.14 ± 0.03	0.17 ± 0.03

Notes:
1. Animals were Dutch rabbits; normal average 1.5 kg and radiated 2.1 kg body weight.
2. Radiated animals received 600 r whole bodyx-irradiation on February 21, 1950.
3. Radiation factors: 250 KV, x-rays delivered at 76.5 r/minute at a FSD of 35 cm., filtered 1.0 mm Al, 0.1 mm Cu inherent and 0.5 mm Cu parabolic.

Project #20023 - Inhalation Studies - Radiopaques

George V. Taplin, M.D., James S. Grevior, M.D. and Philip Noyes

The trinitite implantation studies previously reported in Reports UCLA-158 and UCLA-175 have been continued. The purpose has been to determine whether long term retention of radioactive or stable silica particles produce neoplastic changes at the site of pulmonary contact.

Microscopic sections from animals surviving for 60 days show a definite foreign body reaction to both types of crystals, but nothing specific as a result of local irradiation. The same is true in the group of 10 animals sacrificed after 90 days. Thin sections have been cut and examined by electron microscopy. So far no significant differences are visible in the cells surrounding the imbedded particles.

To determine the amount of absorption of the implanted trinitite particles, the beta and gamma activity in urine, feces and lung and other tissues has been determined in several animals. In no case has it been possible to find evidence of absorption. The activity of removed particles equaled that upon initial implantation.

Project #20026 - Correlation between Biological and Chemical Effects of X-irradiation with Varying Wave Lengths

George V. Taplin, M.D., Jame e S. Grevior, M.D. and Philip Noyes

To augment the studies reported previously (Reports UCLA-76 and UCLA-187) plans have been formulated and equipment prepared to radiate groups of mice with 550 r of γ radiation from our new 100 Curie Co^{60} source. These studies will be completed during the next quarter, after the source has been throroughly calibrated by various methods, one of which has been the use of the chloroform-dye system. The acid liberated from chloroform radiated with Co^{60} is approximately one-third that from exposure to a 250 KVP x-ray beam filtered with Cu, Al and Pb.

BONE DEPOSITION SECTION - Code 91240

Norman S. MacDonald, Ph.D.
Chief of Section

Project #20027 - The Mechanism of Skeletal Deposition of Metallic Ions

Norman S. MacDonald, Ph.D., Florita Ezmirlian, Patricia Spain and Donald E. Rounds

Yttrium: Further evidence of the presence of yttrium within the crystalline lattice of the bone salt of animals injected with YCl_3 was obtained by x-ray diffraction studies with the focusing back reflection camera. In order to prove that the observed lattice distortions were not artifacts arising from the ashing procedure, a study of the changes of interplanar distances of crystals of bone salt with increasing temperature was made. By implanting a thermocouple junction in the powdered bone and recording the temperature continuously during the ignition period., it was shown that the combustion of the organic portion *did not* heat the crystalline portions sufficiently to cause any observable lattice alterations.

Cesium: The growing mice, whose drinking water is a 0.01 molar solution of cesium chloride, are being maintained. Sacrifices are being made at intervals and the femurs preserved for determination of their Cs content. A flame spectrophotometric method is being developed for these analyses.

Strontium: $Sr^{90}Cl_2$ was injected into the yolks of fertile hens' eggs 3-4 days after laying. The eggs were incubated and tibia and femurs removed after

various incubation periods. After mounting, 10 micron sections were obtained for counting and radioautography studies. The purpose of this work is to correlate Sr uptake with the degree of calcification of the embryonic skeleton.

Project #20029 - Agents to Diminish Gastrointestinal Absorption of Cations

Norman S. MacDonald, Ph.D., Florita Ezmirlian, Patricia Spain and Donald E. Rounds

Following completion of the preliminary evaluation of 32 chemical agents (Report UCLA-169), no further work was performed last quarter pending selection and collection of other material to be tested.

RADIOBIOLOGY DIVISION - Code 91310

Andrew H. Dowdy, M.D.
Chief of Division

Leslie R. Bennett, M.D.
Acting Chief of Division

TOLERANCE SECTION - Code 91320

M. A. Greenfield, Ph.D.
Chief of Section

X-Ray Physics Unit - Code 91321

Amos Norman, Ph.D.
Chief of Unit

GENERAL:

Radiation Measurements

M. A. Greenfield, Ph.D., Amos Norman, Ph.D., Paul Kratz, Katherine Hand

The suitability of three types of phosphors for depth dose measurements in a water phantom has been investigated using a 250 KVP x-ray machine as the radiation source. The results are summarized in the following tables in which the response of the phosphors is compared with that of a conventional Victoreen r-meter.

TABLE VIII.

ENERGY DEPENDENCE*

Filtration mm of Cu	Energy above 100 KV %	Ratio of Phosphor Output to Victoreen r Meter Reading: Calcium Tungstate	Zinc Sulfide	Polyvinyl Carbazole
0.00	22	1.0	1.00	1.00
0.26	34	1.5	0.82	1.4
1.1	46	1.7	----	1.6
2.0	62	1.6	0.34	1.7

*Filtration includes 1 mm of Al except at 0 Cu where there is only the inherent filtration of the tube.

TABLE IX.

BACK SCATTER**

Cm of H_2O Behind Probe	Ratio of Phosphor Output to Victoreen r Meter Reading: Calcium Tungstate	Zinc Sulfide	Polyvinyl Carbazole
0	1.00	1.0	1.00
10	0.94	1.2	0.96
20	0.94	1.2	0.96

**Filtration 0.5 mm Cu, 1 mm Al.

TABLE X.

TRANSMISSION*

Cm of H_2O in Front of Probe	Ratio of Phosphor Output to Victoreen r Meter Reading		
	Calcium Tungstate	Zinc Sulfide	Polyvinyl Carbazole
0	1.0	1.0	1.0
10	0.88	1.02	0.93
20	0.85	1.07	0.91

*Filtration 0.5 mm Cu, 1 mm Al

In addition the response of each phosphor was checked as a function of beam intensity and the response was found to be linear within experimental error.

The figures in the last two tables indicate that polyvinyl carbazole is suitable for depth dose measurements, at least up to depths (in water) of 10 cm. It is reasonable to assume, therefore, that other organic phosphors such as anthracene also will be suitable.

Roentgenology Unit - Code 91322

Marta S. Billings, M.D.
Chief of Unit

GENERAL:

A. Radiation Therapy

Marta S. Billings, M.D., Charita Schmitt, Katherine Hand and Louise Burlingame

During the months of December 1951, through February 1952, this department administered x-irradiation to 78 experimental set-ups, comprising 248 single runs and amounting to a total of 376 hours of actual radiation time.

B. Diagnostic Roentgenology

In cooperation with the Project's Health Service 52 chest roentgenograms of employees were taken and diagnosed.

Chest and gastro-intestinal studies were made on 87 rats as part of Project #30030.

Project #30026 - Lethal Dose Determinations

M. A. Greenfield, Ph.D., Marta S. Billings, M.D., A.E. Lewis, M.D., Louise Burlingame

Studies on the effect of total body x-irradiation from two simultaneously energized targets upon the mortality of Dutch rabbits are still in progress. To date the number of animals exposed is too small to draw statistically reliable conclusions.

Project #30030 - Roentgen Studies of X-Irradiation Effects on Gastro-intestinal Motility

Marta S. Billings, M.D., Leslie R. Bennett, M.D., Louise Burlingame

The effect of partial body x-irradiation (450 r) has been studied on 28 mature rats. To date the investigation has been limited to exposure of the upper portion of the body, the field of irradiation extending from the head to and including the inferior border of the sternum.

Barium studies were started one half hour after the irradiation and continued for seven consecutive days. The barium meal was intubated each morning and its progress in the gastro-intestinal tract checked at one hour intervals.

The results show a significant delay in gastric emptying time starting one hour after irradiation and persisting for 78 hours. Some recovery of gastric function is observed on the 5th day and motility appears normal 120 hours after the x-ray exposure.

450 r x-irradiation directed toward the upper half of the body in this rather small group of animals, produced 20-30% less delay in gastric emptying time than that produced by total body exposure. Recovery of normal function was also more rapid after irradiation of the upper half of the body.

Irradiation of the lower half of the body and its effect on gastro-intestinal motility will be the object of further investigation.

RADIATION BIOLOGY SECTION - Code 91330

Raymond L. Libby, Ph.D.
Chief of Section

Bacteriology Unit - Code 91331

James G. Vincent
Chief of Unit

Project #30009 - Bacteriological Aspects of a Toxic Factor Produced by Irradiation Damage to the Intestinal Tract

James G. Vincent, Robert C. Veomett

Experiments are being set up to relate the apparent protection against post irradiation infection afforded by the intestinal lactobacilli to the *in vitro* production of an antibiotic by the intestinal lactobacilli.

Project #30029 - The Production and Isolation of an Antibiotic-Like Agent Produced by a Newly Described Microorganism of the Rat's Small Intestine

James G. Vincent, Robert C. Veomett

With the elucidation of factors governing the *in vitro* production of an antibiotic-like substance by intestinal lactobacilli, yields sufficient for adequate testing seem assured in the near future. The following conditions

have been found to be important to the production of the antibiotic by intestinal lactobacilli:

I. The relation of cell concentration to antibiotic yield:

Other factors being equal, those cultures containing the greatest number of lactobacilli per ml have demonstrated the maximum inhibition of the test organism.

II. Suitable media:

Medium requirements have been found to vary with the strain of lactobacillus studied. Although an excess of some liver extracts brings about a reduction in antibiotic activity, a certain minimum amount of liver is probably required by certain strains of lactobacilli and is not replaceable by the usual substitutes.

III. Period of Autolysis:

Recent experiments in which washed lactobacilli were allowed to stand in an acid buffered solution have yielded samples of consistant antibiotic activity. Other experiments, in which the bacteria were lysed by such methods as freezing and enzymatic digestion, have given negative results. It may be concluded that the antibiotic-like agent is a product of bacterial autolysis.

IV. Relation of pH to Antibiotic Production:

In the absence of adsorbing materials in the cultures of lactobacilli, antibiotic activity is produced at a pH near 4.5. A very low pH, (1.0 or less), brings about a destruction of the antibiotic. The destruction of antibiotic activity in the presence of lactic acid is apparently due to some specific property of the lactic acid molecule, and seems independent of pH.

V. The Presence of Adsorbing Materials:

Since our earlier experiments suggested the practicability of adsorbants for the purpose of increasing the antibiotic yield and since the use of some ion-exchange resins proved unsatisfactory for this purpose, several other possible adsorbing agents have been studied. Calcium salts seemed most promising, and from the compounds considered calcium tartrate was selected for further study because of its low solubility in the pH range of antibiotic production.

VI. The Relation of Temperature to Antibiotic Production:

The highest degree of antibiotic activity has been obtained from those lactobacilli cultures which were allowed an incubation of about two days at 37°C, during which maximum growth is attained. This growth period must be followed by an autolytic period at a temperature below 30°C. Neither the optimum temperature nor the optimum time has yet been determined for the autolytic period.

In the earlier stages of this project, the antibiotic was produced on an agar medium which required an autolytic period of at least one month at room temperature. With the use of calcium salt adsorbants, the length of the autolytic period may be considerably shortened.

RADIATION CHEMISTRY SECTION - Code 91340

Richard F. Riley, Ph.D.
Chief of Section

GENERAL:

MASS SPECTROMETER:

Esther Richter, Richard F. Riley, Ph.D.

The effect of various agents in modifying the uptake of N^{15} glycine by the duck erythrocyte and its incorporation into heme is under joint study with the Hematology Section. The studies on the effect of x-irradiation and nitrogen mustards have been held up during this quarter for repairs on the mass spectrometer and for the preparation of a further supply of N^{15} glycine.

ELECTROPHORESIS SERVICE:

Mary Rotheram

A number of samples of normal rabbit plasma, and plasma obtained at various intervals after whole body irradiation have been submitted to electrophoretic analysis as part of planned studies under Project #30028.

Equipment for ionography on paper is under construction.

Project #30019 - Effect of Irradiation on Protein Metabolism

Esther Richter, Richard F. Riley, Ph.D.

A study of the effect of x-irradiation on the circulating plasma proteins of the rabbit, particularly fibrinogen and lipoprotein, has been initiated. It has seemed of interest to correlate the changes in fibrinogen, measured gravimetrically, electrophoretically and in the ultracentrifuge with changes in lipoprotein, estimated from sedimentation patterns.

Initial trial of the methods involved has been made to establish the best experimental methods. Data obtained so far are too fragmentary to warrant presentation at this date.

Project #30028 - Immunochemical Studies of Ferritin

Mary Rotheram, Richard F. Riley, Ph.D.

The direction of work carried out during the last quarter, in collaboration with the Division of Pharmacology and Toxicology, is described in Project #20025.

During the quarter, a joint report, Report UCLA-180, was issued on the identification of ferritin in the blood of dogs subjected to radiation from an atomic detonation.

RADIATION METABOLISM SECTION - Code 91360

Leslie R. Bennett, M.D.
Chief of Section

Project #30018 - Study of the Effects of Varying Oxygen Tensions on the Response of Mammalian Tissues to X-irradiation

Leslie R. Bennett, M.D., Jerry Flint, Sarah Chastain

Studies to determine the extent of protection given by anoxic anoxia to x-irradiated rat testicular tissue, as described in Report UCLA-175, have been continued.

Further mating of the irradiated male rats with unirradiated females has shown a partial anoxia protection of some animals. After the 33rd day post-irradiation there were no conceptions caused by the males irradiated in 20% O_2. Several of the male rats irradiated in a low oxygen atmosphere, however, have shown a sustained ability to reproduce, and a few have caused occasional fertilizations.

The male rats were each caged with 2 normal females, previously tested for fertility. As each female became pregnant, as indicated by a sudden rise in its weight curve, it was replaced by a non-pregnant female.

Table XI shows the total number and average size of litters for the anoxic and control groups during each 30 day period post irradiation.

Table XI

DAYS Post-Irradiation	Group	Total No. Litters during 30 Day Period	Average No. in the Litters	No. Males Sireing Litters
0 to 30	Anoxic	23	7.7	8
	O_2	20	7.9	8
31 to 60	Anoxic	10	5.4	7
	O_2	2	1	2
61 to 90	Anoxic	9	7.2	4
	O_2	0	0	0
91 to 120	Anoxic	6	8.1	3
	O_2	0	0	0

Project #30023 - Studies on the Late Effects of Roentgen Irradiation

Leslie R. Bennett, Jerry Flint, Rodney Valentine

Studies are continuing on the late effects of irradiation both in rats and rabbits. The "hypersplenic" syndrome described in Report UCLA-154 is being investigated in 2 groups of irradiated rats, 1 group subjected to splenectomy and the second group non-splenectomized.

Project #30031 - Studies on Tissue Inhibitors of Malignancy

Leslie R. Bennett, M.D. Frank Connon, Mary Louise Gouze and Melvin Schoenberg, Ph.D.

During the past quarter the effect of the microsomal inhibitor from the small intestine was studied in the Warburg apparatus. The inhibitor was found to markedly reduce the QO_2 of tissue slices of mouse lymphosarcoma, but to be completely inactive when tested with tissue slices of normal liver, spleen and kidney.

TISSUE TRANSPLANT SECTION - Code 91370

Bennet M. Allen, Ph.D.
Chief of Section

Project #30016 - A Study of the Effects of X-ray Irradiation upon the Hematopoietic Tissue of Tadpoles

Bennet M. Allen, Ph.D., Ole Arne Schjeide and Mary J. Millard

Further experiments and counts have been made in our study of the number of hematopoietic cells in normal and pycnotic condition remaining after different dosages of x-rays in tadpoles killed at different time periods after irradiation. The data are now practically complete and assembled. We hope soon to have this work ready for publication.

The cytological effects of irradiation and colchicine applied separately and in combination has been further examined during the quarter and the following results have been obtained:

1. Application of 100 r + 10 γ or even 500 r + 10 γ colchicine results after 12 hours, in approximately 50% destruction of hematopoietic cells. The combination with colchicine results in considerably increased destruction since 100 r alone produces but 3.3% destruction during this period.

2. 500 r + 1 γ colchicine also produced approximately 50% destruction at 12 hours post-treatment, whereas 1 γ colchicine alone produces only 8% destruction.

3. 100 r + 3 γ colchicine similarly produce the synergistic effect while 100 r + 1 γ colchicine do not.

4. The synergistic effect due to the combined treatment apparently begins at about 13°C. At this temperature part of the tadpoles show extensive breakdown of hematopoietic cells but the remainder do not display the phenomenon. At temperatures below 13°C, i.e. 10°C and 3°C, there is no evidence of synergism.

5. Under conditions of anoxia the extensive destruction due to the two agents does not occur.

6. Recently, extensive early destruction has appeared in some animals injected with colchicine only. Thus whatever factor is active in combination

with colchicine to produce synergism when the animal is x-irradiated may possibly be elicited by other conditions.

7. Since the adrenal glands might be implicated in the observed synergism, cortisone was injected along with colchicine into tadpoles. In our experiments thus far the injected cortisone has not been active in combination with colchicine in producing the synergistic effect. These experiments will be continued.

8. The differences in the mitosis arresting activity of various dosages of colchicine were observed:

a. 20 γ colchicine per 10 gram tadpole produced much pyknosis and some arrest of mitosis. The mitotic arrests were all in the late prophase stage.

b. 5 γ and 10 γ colchicine produced less pyknosis and more mitosis than in the case of 20 γ colchicine per tadpole. However, the sum of mitosis and pyknosis with injection of 20 γ, 10 γ or 5 γ colchicine is the same in each case. The arrested mitoses are all in the late prophase stage.

c. 1 γ colchicine produces very much less pyknosis and the mitotic arrests are in the metaphase which is the usual mammalian type of arrest by colchicine. The sum of pyknosis and mitosis is less than in the case of 20, 10 and 5 γ colchicine.

EMBRYOLOGY UNIT - Code 91371

Ole Arne Schjeide, Ph.D.
Chief of Unit

During the past quarter this new Unit has been added to the Tissue Transplant Section. One of the specific functions of this Unit will be the investigation of the serum and blood cell constituents of the developing embryo and the effect of whole and localized x-irradiation on the presence and formation of these constituents.

RADIATION MECHANISM SECTION - Code 91380

Stanley L. Whitcher, Ph.D.
Chief of Section

Project #30002 - Action of X-Rays on Dilute Aqueous Solutions

Norman R. Todd, Stanley L. Whitcher, Ph.D.

The work with solutions of potassium iodate discussed in the last two quarterly reports has been continued with the following results:

1. The formation of free iodine in the irradiated solutions has been verified. This is in disagreement with some results reported by Clark and Coe (J. Chem. Phys. 5, 97, 1937).

2. In air-saturated acid solutions the iodine formed disappears rapidly but in air-free solutions its concentration remains nearly unchanged for several hours.

3. The amount of iodate reduction was greater in alkaline solution (0.05N NaOH) than in 0.1 N $H_2 SO_4$. The solutions were stable after the exposure and only a trace of hydrogen peroxide was present. The amount of reduction was about 30% greater in air-free than in air-saturated solutions.

4. Additional work on the rate of disappearance of peroxide and regeneration of iodate in the irradiated solutions was essentially in agreement with that reported previously.

5. The kinetics of the reaction between iodine and hydrogen peroxide:

$$I_2 + 5 H_2 O_2 = 2 HIO_3 + 4 H_2 O$$

are being studied to see if this reaction can be used to give an explanation of the post-irradiation effects.

Project #30005 - Action of Radiation on Chemical Compounds Present in Living Cells

Mary Rotheram, Norman R. Todd, and Stanley L. Whitcher, Ph.D.

1. The work done with cysteine and cystine solutions since the issuance of Report UCLA-119 has been summarized and reported in Report UCLA-185. An abstract of this work follows:

When aqueous cysteine solutions are irradiated with x-rays in the range 5,000 - 20,000 r the amounts of cystine found experimentally agree fairly well with those calculated from the decrease in cysteine concentration. With further exposure the calculated values become larger than the experimental and the difference gets progressively greater as the dose increases. The results indicate that disulfide is formed as the first product and with continuing exposure it is itself attacked. This is confirmed by irradiation experiments with solutions of cystine during which a part of it is destroyed and hydrogen peroxide formed in the solution. There is no reduction to cysteine and no production of hydrogen sulfide; accordingly the attack on the cystine is presumed to be entirely oxidative. The oxidation products could not be identified due to the lack of suitable methods of analysis.

2. The survey of radiation-induced changes in the ultra-violet absorption spectra of various compounds is being continued. Results for nucleic acids from two sources are shown in Fig. 2.

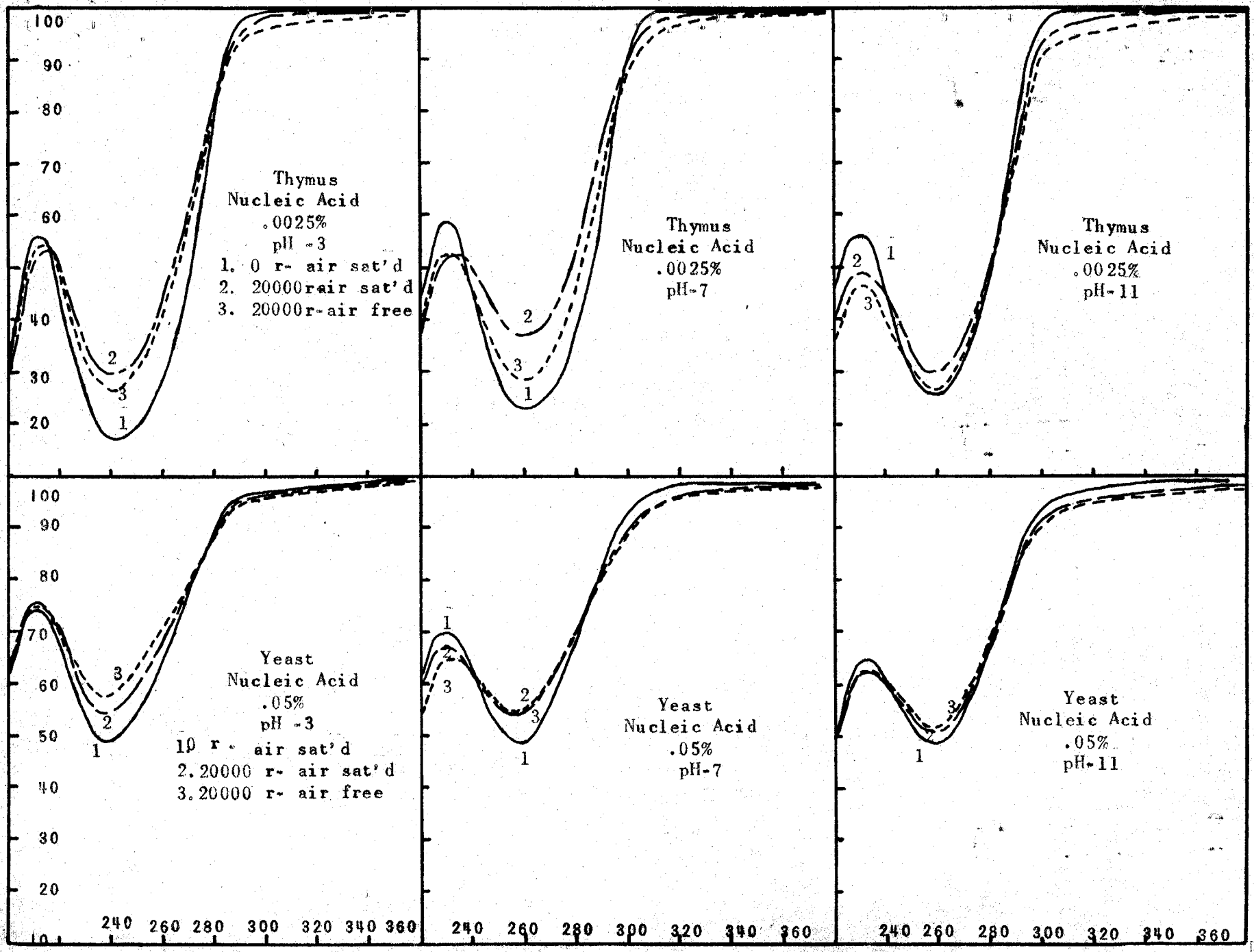

Thymus
Nucleic Acid
.0025%
pH -3
1. 0 r- air sat'd
2. 20000r air sat'd
3. 20000r-air free
Thymus
Nucleic Acid
.0025%
pH-7
Thymus
Nucleic Acid
.0025%
pH-11
Yeast
Nucleic Acid
.05%
pH -3
1. 0 r- air sat'd
2. 20000 r- air sat'd
3. 20000 r- air free
Yeast
Nucleic Acid
.05%
pH-7
Yeast
Nucleic Acid
.05%
pH-11
Per cent Transmission
100
90
80
70
60
50
40
30
20
10
240 260 280 300 320 340 360
Wavelength - millimicrons

Fig. 2

Project #40008 - Old Title: Study of Dust Distribution by Means of Phosphorescent Powders
(New Title: Study of Dust Distribution and Concentration in Inhalation Chambers)

W. C. Burke, Jr. and Leonard Baurmash

In order to aid in the study of the effect of inhaled materials, the distribution and variation in concentration within an exposure chamber must be studied. The chamber under study is a specially designed chamber in which the air flow is axially from the center to the edge. During exposure the animals are slowly rotated on a table inside the chamber. This, theoretically, should average the exposure received by each animal.

The dust feed consists of a sliding table, with dust troughs, moving beneath aspirators which lift the dust from the table and feed it to the incoming air stream.

The chambdr is in the process of being calibrated using silica as the dust. Silica was chosen because it has a density most nearly equal to that of the material to be used in future inhalation studies. The silica is prepared in a Roller Particle Size Analyzer, and the finished product contains particles of ten microns diameter and less.

Originally it was planned to employ a trap in the dust feed system that would remove any particles over ten microsn diameter, and such a trap was made and installed. However, the trap was not found to be efficient. It was decided a mixing chamber that would aid in breaking up aggregates was more desirable. This chamber was installed, and it has proved satisfactory.

The air for the dust feed system was supplied by a rotary type pump. This air source was found to be entirely unsatisfactory due to the difficulty of filtering out entrained oil and inadequate capacity. A pressure of at least 20 psi is necessary to prevent the lines carrying the dust into the inhalation chamber from choking up. The rotary pump could not supply this demand, so it was necessary to use the Project air supply.

Before any calibration work was done, a zinc-cadmium sulfide phosphor was fed into the chamber through the dust feed mechanism. Ultraviolet light was

then played over the inner surface of the chamber. The air velocities and directions within the chamber at numerous points were then measured with an Agitair meter. These studies revealed that there were no dead air spaces, and that the dust was evenly distributed throughout the chamber.

Calibration of the air flow through the chamber was done by inserting the measuring wand of the Agitair Meter into the duct leading from the chamber to the exhaust blower. By means of a by-pass valve in the exhaust system, the air flow through the chamber may be regulated. Measurements of air velocities were made at the middle and at the side of the duct for various air flow rates. The results were as follows:

TABLE XIII

Manometer Reading in inches of H_2O	Middle ft/m	Side ft/m	Avg ft/m	Ft^3/m
3.2	4450	4200	4325	211
3.0	4180	4100	4140	202
2.5	3890	3810	3850	188
2.1	3670	3500	3585	175
1.6	3170	3100	3135	153

Filter papers are used for the dust concentration calibration. These filter papers are very sensitive to humidity; and since it was desirous to minimize any weight error due to adsorbed moisture on the filters, a dry box was constructed. This box is large enough to hold a beam balance, and it has a pair of rubber gloves attached by which things in the box may be manipulated. Sufficient drying agent is placed in the box to last a week. Fresh numbered filters and the filters from the days' run are placed in the box each evening, and all weighing is done the next morning. Thus, plenty of time is given for conditions in the box to reach equilibrium, and ooening of the box is kept at a minimum.

Calibration of the chamber consists of placing a weighed amount of silica in one or more troughs of the dust feed mechanism. This dust is fed into the inhalation chamber where it is mixed with the air in the chamber. A measured volume of air from the chamber is drawn through a clean, weighed filter, any dust in the air being deposited on the filter. This filter is then dried and weighed, the difference in weight being the dust weight. Eight half-hour dust samples are taken for each run. By this means the dust concentration in the chamber can be determined for each adjustment of the feed mechanisms. Preliminary checks indicate a variation of < 10% for each of the settings which were tested.

Upon completion of calibration studies, this material will be reported in a forthcoming UCLA report.

Project #40020 - Research into and Development of Equipment to Aid in the Quantitative Measurement of Microscopic Particulate Matter by Electronic Means

Leonard Baurmash and W. C. Burke, Jr.

To assist in the detection of minute amounts of alpha activity, work is being continued in developing an extremely low background. At the present time, the work is progressing along the lines of lowering the electronic noise in the system. An amplifier and scaling circuit has been developed which is noise free in the range at which it is being used. An attempt is being made to reduce the tube noise to acceptable levels. An electronic method has reduced the noise to less than one count per hour. More work will be done to reduce other interfering circuit noise.

HEALTH PHYSICS SECTION - Code 91430

Louis B. Silverman
Chief of Section

1. General Health Physics

A. The data and results compiled from the Project's general area monitoring and personnel monitoring programs indicated that none of the personnel at this Project have received overtolerance exposures during this period.

B. Special monitoring of two of the Project's personnel who went to the Nevada Test sites for soil samples from the crater areas indicated that one man had received a radiation exposure maximum of 1700 mrem for the one week trip and the other man had received a maximum of 1459 mrem for the same period. Both of these individuals had received a total radiation exposure of only 10 mrem during the eight week period prior to the trip, and since the trip (a period of seven weeks to date), they have received a total of < 20 mrem. Results of the urine activity analyses on these individuals are described later in this report under Project #40014.

C. The off-Project film badge services for the North American Aviation Company's Atomic Energy Research Project was continued during this period. A total of 1220 film badges were supplied and processed. The results indicated that only one person had received an overtolerance exposure. This was 1.6 times the tolerance level (300 mrem per week) for one badge period. The probable cause was investigated and corrected by that Project, since subsequent badges of this individual are < 0.1 of tolerance.

D. The health physics research program has again been curtailed due to the change of personnel in this section during this period. This decrease in the research effort was necessary in order that the necessary health physics services and monitoring programs could be continued while the replacement was being trained.

II. Health Physics Projects

Project #40011 - Photographic Dosimetry

L. B. Silverman, Arturo Frisoli

A. Research Program

1. The new plastic tape film badge holder developed in this section has been produced and placed in service on this Project. It has proved in operation to have accomplished the objectives set for this new holder, and in addition, it has increased the efficiency of the film badge monitoring operations by reducing the man hours necessary for preparing and processing film badges. Another advantage has been the decrease in the number of lost film badges to zero since the initiation of the new holder. More complete details of design, specifications, methods of assembly and use of these holders are described in Report UCLA-189.

2. A special series of 32 Eastman Type K films varying in size from 1¼" x 1¾" to 14" x 17" were used to cover the entire outer surface of the Co^{60} chamber so as to measure the radiation coming through the shielding with the 100 curie Co^{60} source in the working position. Special calibrations for the various emulsions used were done so that proper dosage interpretations could be made from the density readings. The results indicated that the design of the shield was good in attenuating the radiation from the large Co^{60} source to an average safe level of approximately 1 mr/hr at the shield's surface. More complete details of design, construction and operation of this shield are described in a report now in preparation in cooperation with Dr. Greenfield of X-Ray Physics Unit and Mr. Dickinson of the Shops Section.

3. A series of calibration tests have been done to compare Dupont's new type 558 film packet with our standard Eastman type K films. Final conclusions of results await further information now being obtained.

B. Film Badge Dosimetry

L. B. Silverman, Phyllis Winnard, Richard K. Dickey

The regular normal film badge monitoring program was continued during this period. A total of 162 people and 18 locations were monitored. Of this total 9 persons were also monitored with wrist badges. The maximum exposures on Project during this period was < 0.2 times tolerance for two persons during one exposure period.

With the completion of the new plastic tape film badge holder this monitoring program has been expanded to include *all* Project personnel on a two-week exposure period. The new holder with its ease of use and x-ray identification marking of the films has made the expanded program possible without increasing the work load of this Unit.

Project #40012 - Sea Burial for Active Wastes

L. B. Silverman, R. K. Dickey

Ten 55 gal drums were prepared and buried at sea. They contained various long-half-life active wastes and contaminated soils. The method of preparation varied with the type of wastes. Relatively low level wastes were loaded into the drums and the drums capped with approximately two to three inches of concrete. Relatively higher levels of β and γ active wastes were loaded into a drum lined with approximately three inches of concrete and then capped with three inches of concrete.

Project #40014 - General Urine Activity Analyses

L. B. Silverman, R. K. Dickey

Five series of urine activity analyses were done during this period.

1. A series was done on three individuals involved in a minor spill while working with Sr^{90}. The results of these analyses indicated that *no* Sr^{90} was being excreted in the urine of these persons.

2. A background control series was done for polonium activity on an individual planning to work with a multimillicurie polonium source. This individual also had worked with Po^{210} sources prior to being employed by this Project. The results indicated that *no* Po^{210} was being excreted in his urine.

3. A routine series of runs for fission products were done on the two persons that had gone to the Nevada Test sites to collect soil samples. The results indicated that neither of the men were excreting any detectable amounts of fission products.

4. A routine series of analyses was done on two persons with the highest exposure potential with long-lived isotopes. The results indicated that *no* activity above the normal background level was being excreted in the specimens analyzed.

5. A series of Sr^{90} analyses were done as a courtesy consultation for two individuals of the Lockheed Aircraft Company who were involved in an accidental spill of Sr^{90} contaminated powder. This spill points up the fact that so-called "safe radiation sources" must be checked periodically for their ability to contain the radioactive material. In this particular case, the Sr^{90} was incorporated into a plastic plaque and the plastic material apparently crumbled and powdered producing a highly dangerous contamination hazard. Fortunately the men involved recognized the hazard immediately and their action prevented the wide spread of the contmaination. Their urine specimens were collected and submitted to this Project about one month after the accident had happened. The results of the analyses indicated that *no* Sr^{90} was being excreted in the specimens analyzed.

Project #40015 - Hood Efficiency Survey

L. B. Silverman

A general ventilation problem in one of the laboratories in the Hematology Section was investigated and recommendations are being acted upon by that section.

Project #40016 - Air Monitoring

L. B. Silverman, R. K. Dickey

1. Two runs were made in two separate laboratories where the Alamogordo Section are preparing Nevada Test site soils and fission products spiked soils for their experiments. Both runs indicated that no activity above normal background after decay of natural radon and thoron products.

2. A series of activity determinations made on rainwater since the atomic detonations in Nevada last fall indicated no abnormal activities in the rainwater.

Project #40017 - Development and Maintenance of Project Rules

L. B. Silverman

Special operating instructions for the safe use and operation of the 100 curie Co^{60} source in its irradiation chamber shield were drawn up and placed in effect. In connection with these rules an indoctrination program was supervised for the training of the personnel authorized to use this new high intensity radiation source.

Project #40018 - Decontamination Problems

L. B. Silverman, Arturo Frisoli, R. K. Dickey

1. Two problems involved decontamination of the fission products from (a) an aluminum table jig and (b) a cast iron rotating table. The decontamination was accomplished with Versene and was 100% successful in each case.

2. One problem posed by a Sr^{90} solution spill was simplified by the protective nature of the Kimpack "diaper paper" covering on the bench and in the hood.

3. The dissemination of decontamination procedures and information was continued to persons requesting and/or in need of this service.

Project #40037 - Health Physics Applications of the UCLA Alpha Scintillation Counter

L. B. Silverman

No formal activity on this project during this period except discussions of future plans for several types of experiments.

Project #40043 - Effect of Contact Trinitite Exposures on the Skin of a Rabbit

No activity on this project during this period due to circumstances mentioned at the start of this report.

III. Miscellaneous

A. All high range survey meters were calibrated during this period.

B. Special monitoring jobs during this period have involved:

1. Complete monitoring of a new research program by the Biochemistry Division involving the use of C^{14}.

2. Special monitoring of the storage and use of the 100 curie Co^{60} source.

3. Special monitoring of the use of 20 mgm radium and 5 mc P^{32} sources by the X-Ray Physics Unit.

4. Special monitoring of the Alamogordo Section's Field Party personnel on their last trip to the Nevada Test site for soil smaples was covered above. However, the arrival of these soil samples on Project involved special monitoring operations of the truck and the transfer of samples to their storage location in yard area #2 behind a sandbag barricade. It also involved a special survey of all the film storage locations in the third wing of the Project due to increased background levels. As a result of these surveys an additional layer of sandbags was recommended and placed for the needed shieldings.

C. The Colorimetric Dosimetry Section was assisted in the preparation of several mc P^{32} sources for their use during this period.

D. Courtesy consultations and special health physics instructions were given to various off-Project individuals and to members of the California State Board of Health and Los Angeles City Health Department.

E. A series of Health Physics Seminars were given to the Project personnel during this period.

IV. General Safety

Copies of the first rough drafts of the General Safety Section and the Fire and Disaster Plan Section of the Safety Manual were distributed for circulation within each division of the Project. Corrections, additions, criticisms, and comments were solicited. The pertinent corrections will be incorporated in the final draft of this Manual.

ELECTRONICS SECTION - Code 91440

William R. Kennedy, Ph.D.
Chief of Section

GENERAL

William R. Kennedy, Frank C. Strebe, Jacob E. Dietrich, John B. Hall, Manuel Alvarez, Harry W. Hazell

This section has continued its work in the instrumentation for other Project laboratories, the maintenance of Project electronic equipment, and research and development.

A prototype of an electronic system to help safeguard personnel during the use of the Co^{60} source has been completed and is ready for final engineering and instrumentation. Measurements have been made with a scintillation tube and a D.C. amplifier which operates a relay to determine the position of the Co^{60} in the lead housing. Tests have shown that the position of the source can be determined by means of the scintillation tube which with its associated circuit turns on a red or a green light to indicate to the operator whether or not the radiation chamber can be entered.

Numerous small instrumentation problems have been taken care of for various Project laboratories during this period. These have included adaption of scaler equipment to various types of G-M tubes, fabrication of junction boxes for electronic equipment and safety devices for protecting G-M tubes during periods of electrical breakdown on the Project. Work has continued on various research and development projects, and these are discussed in some detail in the following.

Repairs were effected on various types of Project electronic equipment.

Project #40034 - Telemetering Installation

Frank C. Strebe, William R. Kennedy, Manuel Alvarez

During a part of this quarter, it was necessary to discontinue the monitoring of the atmosphere because part of the equipment was affected by the heavy precipitation. The G-M tubes for both the telemetered information system and for the scaler mounted at the transmitter site were rendered inoperative due to high voltage leakage. Part of this difficulty may be due to exposure of the coaxial line, which carries the high voltage, to the rain. The construction of the G-M tube housings were such that the high voltage terminals could break down in excessive moisture conditions. Steps are being taken to remedy this situation by properly sealing the G-M tubes into moisture proof containers and by possibly drilling holes into the concrete roof of the building housing the telemetering transmitter so that the coaxial lines can be run directly into the cupola from below instead of the roundabout manner necessary at the present time.

All of the receiving equipment has been incorporated into a rack and panel which has helped considerably in consolidating this equipment.

The system for transmitting count-rate data instead of individual counts is progressing satisfactorily. An AM-FM system is being investigated for this purpose. The circuit under development consists of an audio oscillator, a rate meter, a difference amplifier, and a phase inverter. Out of phase voltages from the audio oscillator are apllied to the two grids of the difference amplifier. At normal background count-rate the difference amplifier output is adjusted to zero. The output from the rate meter circuit controls the gain of one side of the difference amplifier. The other side of this amplifier is used as a reference. The net result is an audio signal output whose amplitude is proportional to the background count rate.

A general report is being prepared on the present system in which the details of this system are discussed as well as results from the several tests are presented.

Project #40050 - Semiconductors as Crystal Counters

Frank C. Strebe, William R. Kennedy, Ph.D.

No work was done on this project during this quarter. However, with better amplifiers available than heretofore, it is expected that this project will be activated shortly.

Project #40051 - Special Purpose Vacuum Tube Laboratory

William R. Kennedy, Ph.D., Harry W. Hazell

The vacuum system for tube processing is now being assembled. Various pieces of glassware have been constructed for use with a commercial oil diffusion pump. An ionization gauge amplifier has been built and tested for use with the system.

Further work on the gaseous subminiature scaling tubes was set aside during this quarter. However this will be started again shortly.

Several tubes of interest to this field have comt to our attention and we expect shortly to be able to take advantage of the facilities now available to develop such tubes.

Project #40052 - Subminiaturizing Scientific Electronic Equipment

John B. Hall, Jacob E. Dietrich, Fred A. Bryan, M.D., William R. Kennedy, Ph.D.

The development of a subminiature scaler is progressing satisfactorily and is rapidly approaching the final assembly stage.

Three decade units with a total scaling factor of 1000 have been constructed and tested, and have been found to be satisfactory.

At the present time the amplifier and trigger circuits have been designed and a prototype tested. The final layout of this part of the scaler as a complete unit is being accomplished. It is expected that this unit can be enclosed in a case of dimensions 4-1/2" x 3" x 1".

Development work on both the high voltage and the B+ power supplies is almost completed. The B+ power supply makes use of a doubler circuit with selenium rectifiers. This supply has been experimentally tested and is now ready for final assembly. Two high voltage supplies were developed around radio frequency oscillators to operate either a scintillation detector or a Geiger-Mueller tube. One used a modified Colpitts oscillator circuit and the other a separate feedback grid coil circuit. The latter was adopted for this instrument because the components were more readily available to us. This supply is electronically regulated by a degenerative amplifier. The voltage is variable from 500 to 1800 volts and can furnish sufficient current to operate a scintillation detector tube. The regulation is within 1/2 of 1%. An aluminum case 4" x 2-5/8" x 3-1/2" completely contains the oscillator and electronic regulator.

Tentative data now indicates that the complete scaler can be housed in a case approximately 10" x 6" x 6". This case will also contain the scintillation detector tube and the necessary sample holder.

SPECTROSCOPY SECTION - Code 91450

Ralph E. Nusbaum, Ph.D.
Chief of Section

Light Spectroscopy Unit - Code 91451

George V. Alexander
Chief of Unit

I. Emission Spectroscopy

George V. Alexander, R. C. Barbera, R. E. Nusbaum, Ph.D.

In the last report the spectrographic analysis of rat liver ash by a solution method was described. In this case the analysis was made for the strontium content. It was pointed out that the method would be applicable to certain other tissues. During the present period preliminary work has been done to extend this method to include other elements, for example lead, bismuth, and silver. As indicated in Report UCLA-175, the liver samples are wet ashed and the ash is taken up in 7N HCl. The procedure is used without a buffer. Standard samples are prepared by adding known amounts of the elements to be determined to solutions of the ash of normal liver. Lead and Silver are detected in the normal. In these cases, it is necessary to determine spectrographically the residual amount by an addition technique. In the case of the

elements not detected in the normal tissue, the standards simply contain the amounts of these elements added when the standards are prepared.

Work has been continued on a procedure for the analysis for trace elements in blood. Sodium and potassium have been satisfactorily removed from the blood by ion exchange techniques. This has greatly increased the sensitivity to trace elements. Further work along these lines will be directed toward establishing the general location of these trace elements within the blood.

A number of miscellaneous qualitative and quantitative analyses have been made of materials used in the research work of this project.

Project #40053 - The Use of Controlled Atmosphere for Spectrographic Excitation Sources

R. E. Nusbaum, Ph.D., R. C. Barbera, George V. Alexander

This project was established within the past quarter for the design, construction and application of a controlled atmosphere spectrographic excitation housing to be used in the analysis of samples encountered in the work of this unit. At the present, the project is in the preliminary design stages.

II. Infrared Spectroscopy

R. C. Barbera, George V. Alexander, R. E. Nusbaum, Ph.D.

A number of records of infrared absorption spectra have been prepared for samples submitted by the Biochemistry Division. Several spectra of serum samples have been prepared for the Embryology Unit of the Radiobiology Division employing the *net* technique described in the last quarterly, Report UCLA-175.

X-ray Diffraction Unit - Code 91452

George V. Alexander, R. E. Nusbaum, Ph.D.

The powder patterns of several miscellaneous materials were obtained and used in conjunction with the emission spectrographic analysis to identify the material. X-ray diffraction patterns have proved quite helpful in following the progress of the concentration work being done on blood by this section.

In addition to this work, there have been x-ray diffraction studies conducted by the Bone Deposition Section as a part of the investigation of the crystal structure of bone salts.

ELECTRON MICROSCOPE SECTION - Code 91460

F. W. Bishop
Chief of Section

S. B. Elliott

A heavy load of routine materials was examined in the electron microscope

during this period. A lyophilizing apparatus for the preparation of tissues for thin sectioning was completed and is under test. A direct-viewing device adapted from a Spencer stereoscopic shop microscope was installed on the electron microscope (Fig. 3). It represents a considerable improvement over the standard RCA focussing magnifier inasmuch as it affords a much wider field of view and somewhat more illumination. It also does not need to be moved in and out of the field of view but remains in viewing position at all times. The stereoscopic feature and the position of the device makes the viewing of the final screen much less fztiguing than the previous monocular arrangement. The simple magnifier usually mounted on the front of the microscope was installed on one of the side windows.

The use of permanent knife backs as recommended by Hillier (*Rev. of Scientific Instruments*, 22: 185-188, 1951) has overcome many of the difficulties encountered in the sharpening of microtome blades, and this plus general improvements in fixing and embedding techniques has resulted in satisfactory thin sections for the study of fine detail in tissue. Many hundreds of sections were ut and examined for other divisions of this Project. The substitution of an alcohol mixture for dioxane as the liquid medium in the section-collecting boat has greatly reduced the difficulties due to rusting of the blade.

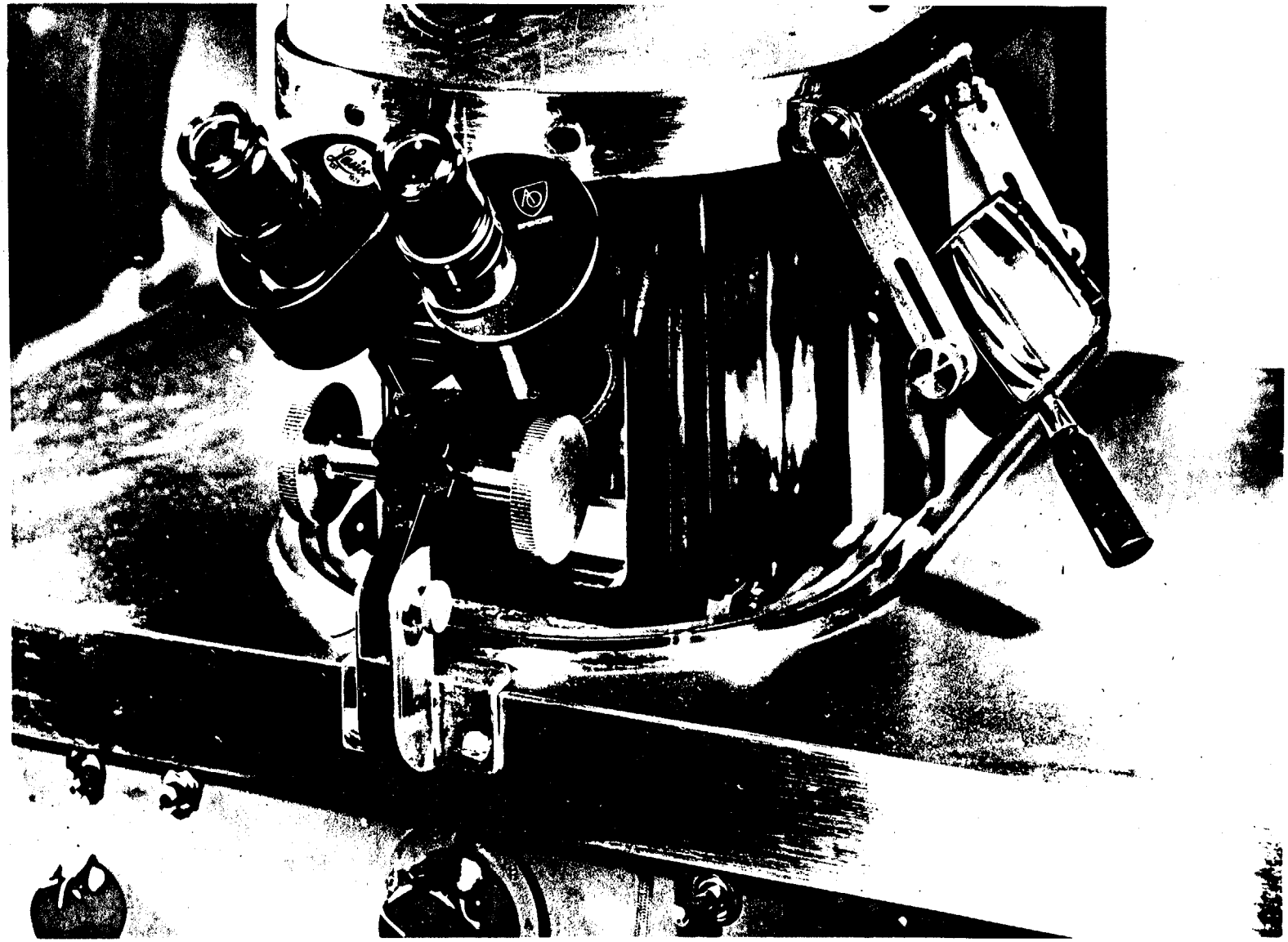
COLORIMETRIC DOSIMETRY SECTION - Code 91470

George V. Taplin, M.D.
Chief of Section

Project #40046 - Development of a Colorimetric Personnel Dosimeter

George V. Taplin, M.D., Clayton H. Douglas, Sanford C. Sigoloff

During the last quarter, the activities of this section have been directed mainly toward the practical solution of the problem of rate dependency in the chloroform-dye method. Progress has been speeded by the availability and completion of the new 100 curie Co^{60} source. The major radiation characteristics of the chloroform-dye system have been restudied, using pure chloroform produced by fractional distillation deecribed previously in Report UCLA-175. With this method, it has been possible to produce chloroform samples of identical radiation sensitivity from various lots and brands of reagent grade chloroform. It has been demonstrated that rate dependency may be almost completely resolved by adding ethyl alcohol in optimum concentrations (1.0 - 2.0 percent by volume). Dosimeters have been prepared, using various percentages of alcohol, and have been tested for heat and light stability, rate and energy dependence, as well as for sensitivity to x and gamma radiation. Also, the reproducibility and accuracy of the methods of filling and sealing have been determined by spectrophotometric analysis and by final calibration on the standardized 300 mgm radium source.



Viewing Devices Mounted on the Electron Microscope

Fig. 3

In addition to the basic studies, several series of dosimeters have been prepared for special purposes.

1. To extend the energy dependence studies into the high energy range using the Chicago betatron operated at effective energies varying between 3 and 5 mev.

2. To obtain independent evaluation of the radiation characteristics by other investigators.

a. Dr. G. Failla's group.

b. The Army Chemical Corps, Chemical and Radiological Laboratories.

c. National Bureau of Standards

The results of recently performed basic studies on the radiation characteristics of the pure chloroform-alcohol-dye system follow:

1. Energy Dependence Studies

Data shown in Fig. 4 demonstrate the effect of varying the energy of x and gamma radiation on acid production from chloroform stabilized with increasing concentrations of ethyl alcohol. The rate of irradiation was held constant at 12 r/min with the x-ray studies. It is apparent that the system is highly energy dependent, particularly within the low energy x-ray range. However, a plateau is reached at energies exceeding the mean value from radium. In addition, it is again demonstrated that ethyl alcohol reduces acid production from chloroform by a factor of 20^{+}.

2. Rate Dependency Data

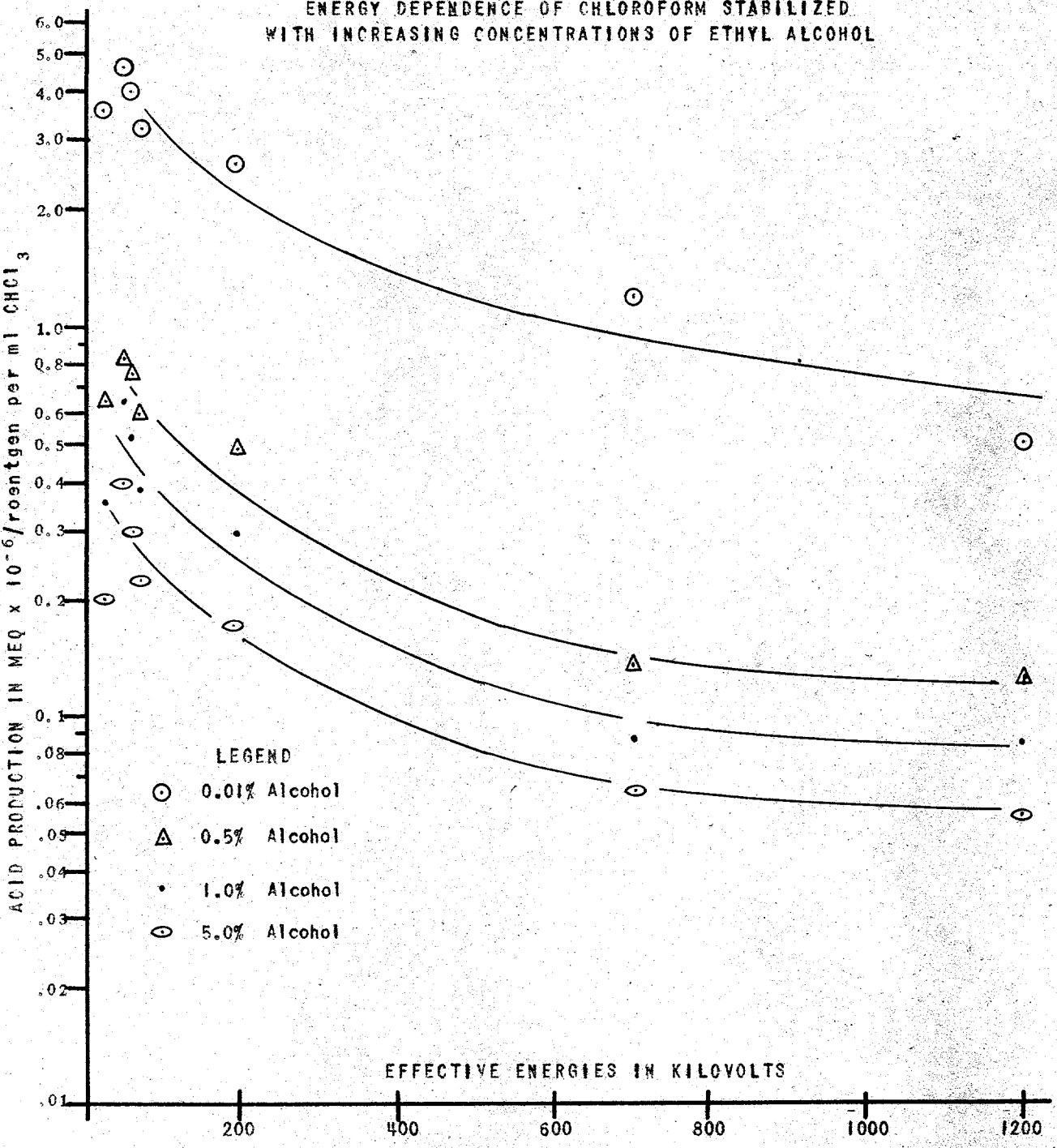
In Fig. 5, the effect of wide variations in radiation intensity on acid evolution from chloroform stabilized with increasing concentrations of ethyl alcohol is presented. It is shown that when the alcohol content in chloroform exceeds 1-2%, rate dependency is resolved. More important, when radiation rates exceed 800 r/min, acid production/r is resolved for chloroform preparations containing various concentrations of ethyl alcohol.

3. Temperature Dependence Studies

When the temperature of the chloroform-dye system during irradiation is below or above room temperature, acid production is altered. More acid is formed at high temperatures, less at low values. Furthermore, as the alcohol content is raised, temperature variations during radiation play a less important role. See Fig. 6.

4. Calibration Studies - Co^{60} Source

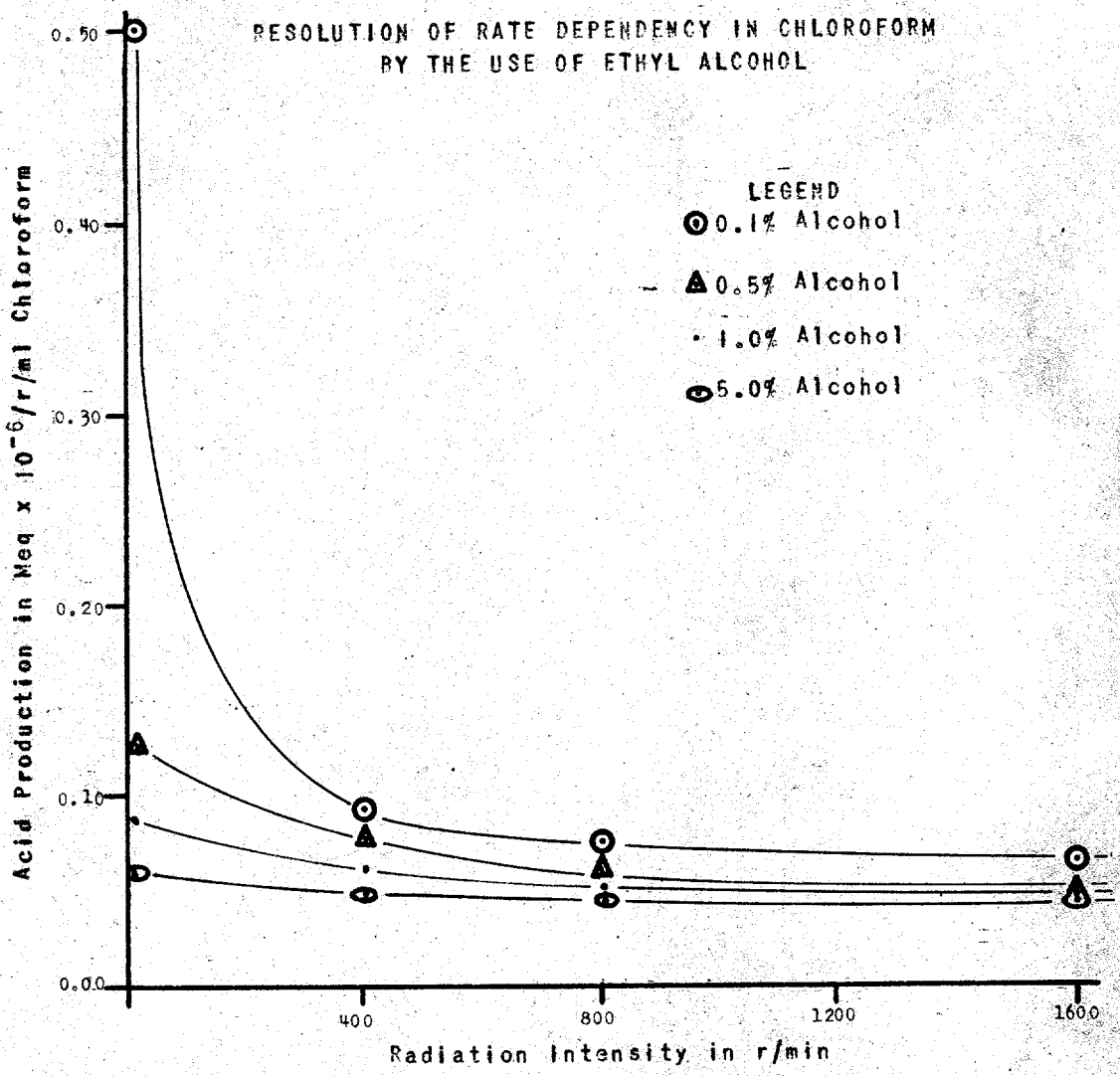
The Co^{60} source shield has been completed recently and has been thoroughly tested regarding health safety features under operating conditions. This work has been done by the Health Physics Section of the Division of Industrial Hygiene. Film badge data on the efficiency of the shield indicate that it is perfectly safe to work full time in the room with this equipment. The mechanical safety features embodied in the design of the shield have been found



Notes:

1. All samples radiated in quadruplicate in 2 ml pyrex glass stoppered volumetric flasks.
2. Each sample contained 1 ml chloroform and 0.5 ml dye.
3. Radiation Sources a) 85 KV Picker Portable Field X-Ray Unit
   b) 250 KV Picker Industrial X-Ray Unit
   c) 300 mgm Standardized Radium Source
   d) 96.4 curie Co^{60} Source
4. Effective Energies from sources described in (3) a) 40 KV b) 45, 50, 60, and 190 KV c) 700 KV d) 1200 KV

Fig. 4



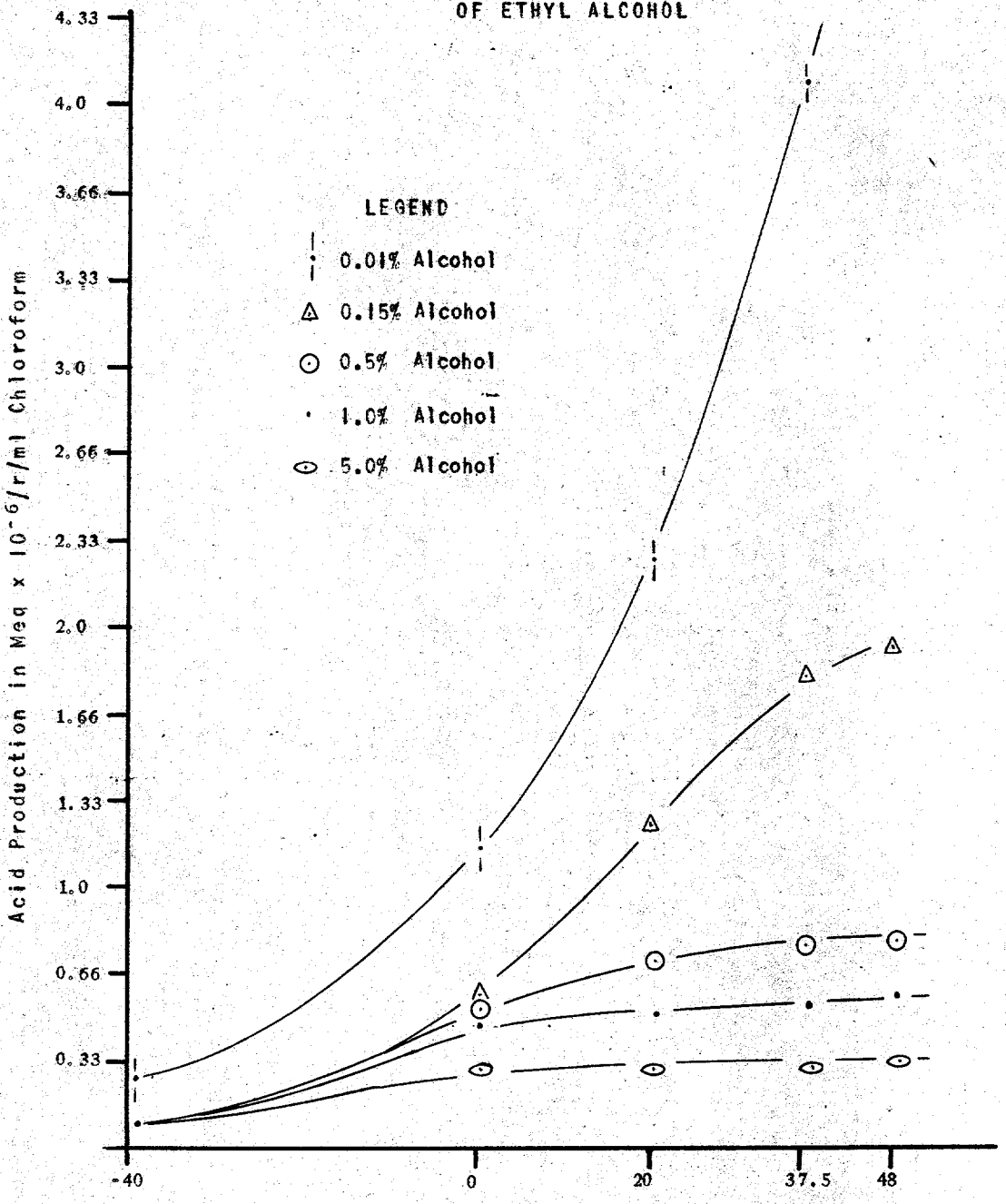
Notes: 1. Radiation Sources: a. 300 mgm radium - 5 r/min
b. 96.4 curies Co^{60} - 400-1600 r/min

2. Samples radiated in 2 ml pyrex volumetric glass stoppered flasks.

3. Each point represents the mean of six separate determinations.

Fig. 5

EFFECT OF VARYING THE TEMPERATURE OF CHLOROFORM DURING X-IRRADIATION ON ACID PRODUCTION FROM SAMPLES STABILIZED WITH INCREASING CONCENTRATIONS OF ETHYL ALCOHOL



Notes:

1. Each point is the mean value computed from 4 separate determinations.
2. All samples were radiated in flame sealed pyrex tubes.
3. Each sample contained 1.0 ml chloroform and 0.5 ml dye.
4. All samples were irradiated with the 250 KVP X-Ray Unit, operated at 15 ma at a rate of 76 r/min. The beam was filtered with 0.25 mm Cu and 1.0 mm Al.
5. Samples were exposed in a water bath with controlled temperatures.

Fig. 6

entirely satisfactory. However, an added safety device is also employed routinely as an accessory safety precaution. The involves the use of a beta gamma survey meter, during the opening of the exposure chamber door, to be doubly certain that the source is in the safe position.

a. Determination of the position of the source in its holder in respect to the center of the field.

1. Values in A/r with dosimeter holder stationary

2. Values in A/r with dosimeter holder rotating (2 rpm) see Table XIV.

b. Linear relationship between A/r from chloroform, and dosage computed according to the inverse square law using 96.4 curies of Co^{60}. See Fig. 7.

Table XIV

RADIATION DATA DEMONSTRATING THE ACCURACY OF THE POSITION OF THE Co^{60} SOURCE IN RELATION TO THE SAMPLE HOLDER

Sample Holder	Trials	Segments of the Sample Holder: Percent Deviation from Mean Value of Acid/r 1st Quarter	2nd Quarter	3rd Quarter	4th Quarter
Stationary	1	+5.8	+2.2	-3.5	-5.5
	2	+5.1	+2.2	-5.2	-2.3
	3	+8.2	+2.8	-11.0	0.0
Rotating at 2 rpm	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0

Notes: 1. Each value represents the mean of 6 separate determinations

2. Each sample contained 1.0 ml chloroform stabilized with 5.0% alcohol and overlayered with 0.5 ml dye.

3. Dosage administered all samples was 2000 r at 400 r/min.

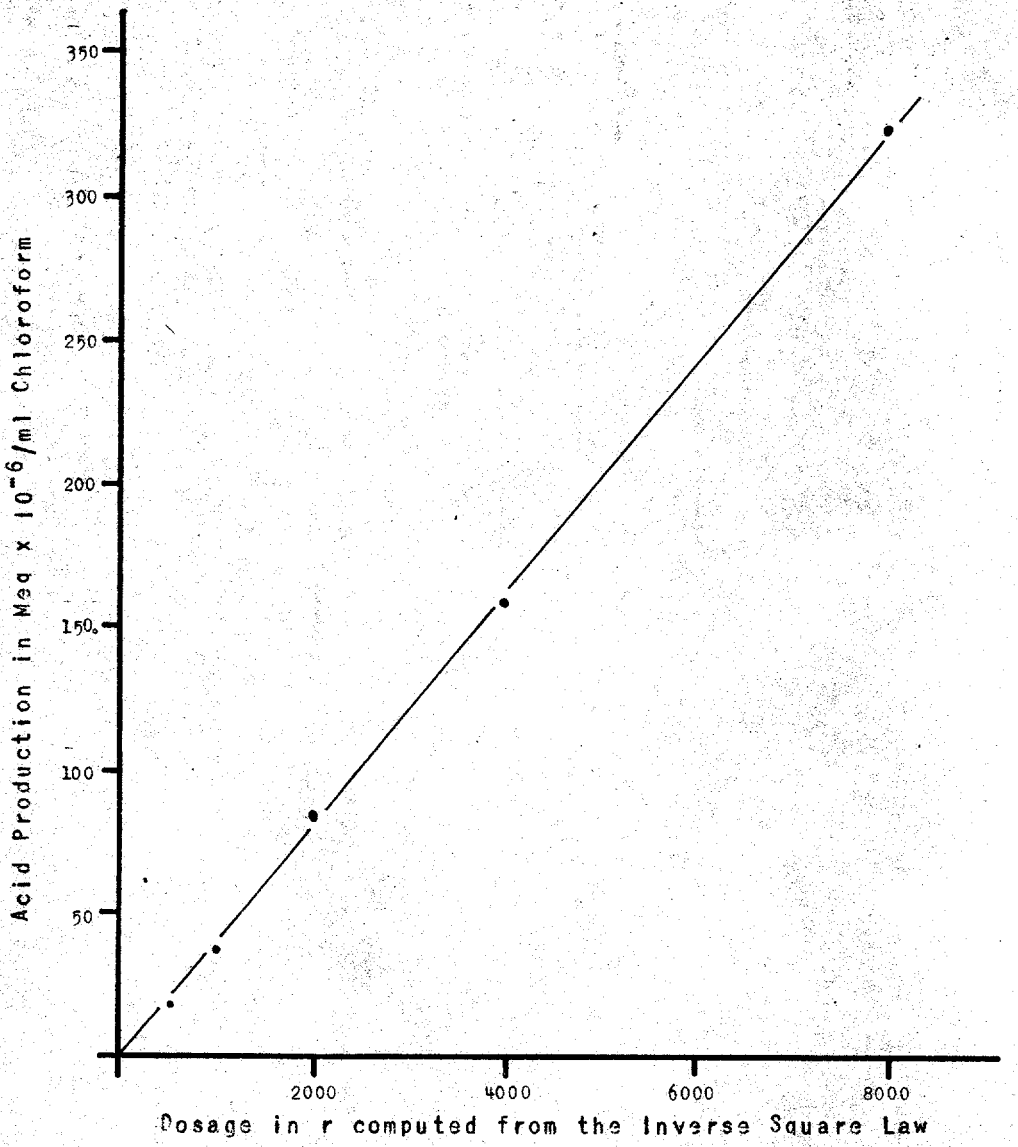
5. Quantitative Evaluation of After Reactions in Radiated Chloroform-dye Samples

One of the characteristics of a chain reaction is the occurrence of a continuation of thd reaction following the period of irradiation. For some time, it has been grossly apparent that samples of chloroform, washed free of alcohol, manifest an after reaction. To quantitate this phenomenon and to determine the amount of alcohol necessary to abolish it, appropriate studies have been made. A large series of chloroform-dye samples is irradiated and acid production is determined by titration at specified intervals. See Fig. 8 for data which demonstrate that a significant after reaction occurs during the first 30 minutes post-irradiation, and that this phenomenon is abolished by the addition of minute amounts (0.10%) of ethyl alcohol to the system.

6. Reaction Mechanism Studies with the Chloroform-Dye System

By plotting the data shown in Fig. 4 on log log paper (See Fig. 9) it is

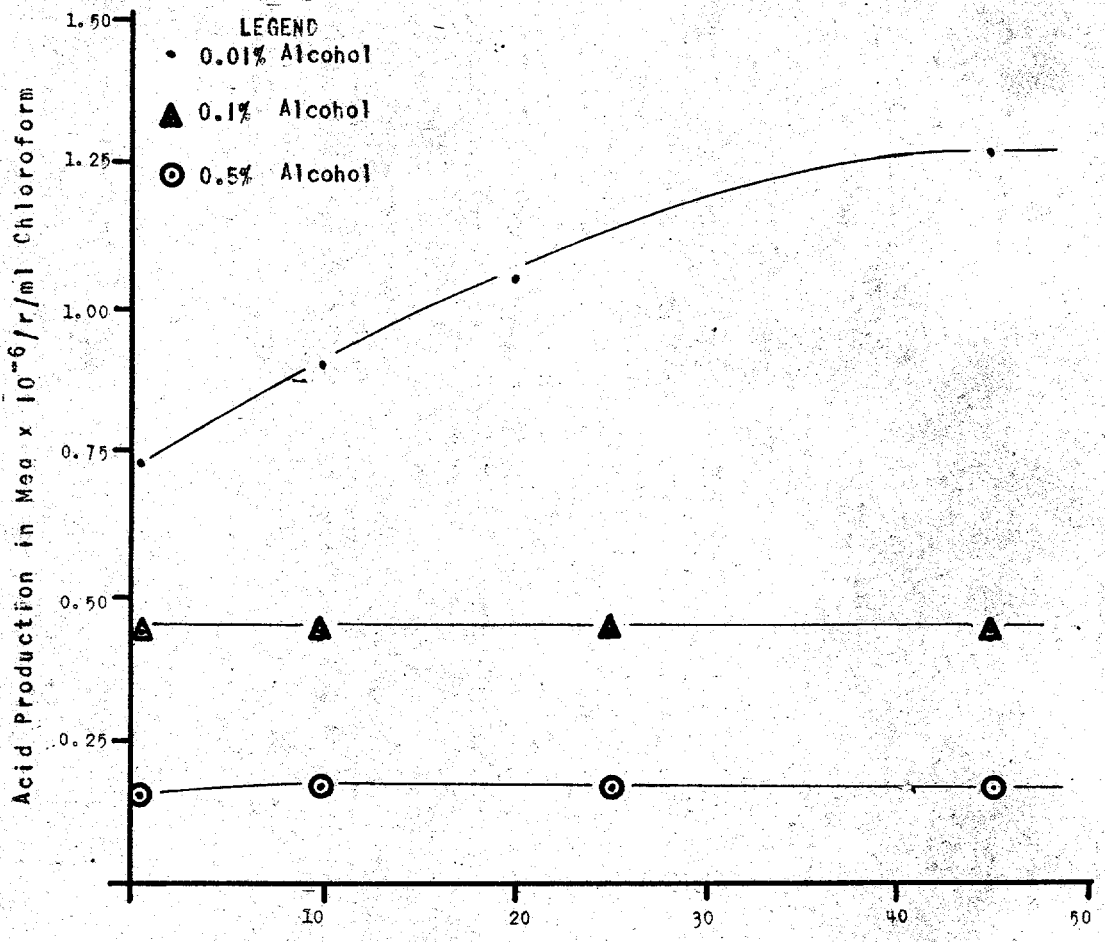
LINEAR RELATION BETWEEN ACID PRODUCTION
FROM CHLOROFORM AND DOSAGE OF Co^{60}
GAMMA RADIATION



Notes:
1. Values at each point represent the mean of six separate determinations.
2. Each sample contained 1.0 ml chloroform stabilized with 5.0% alcohol and overlayered with 0.5 ml dye
3. Doses computed from the Inverse Square Law, using 96.4 curies Co^{60}.

Fig. 7

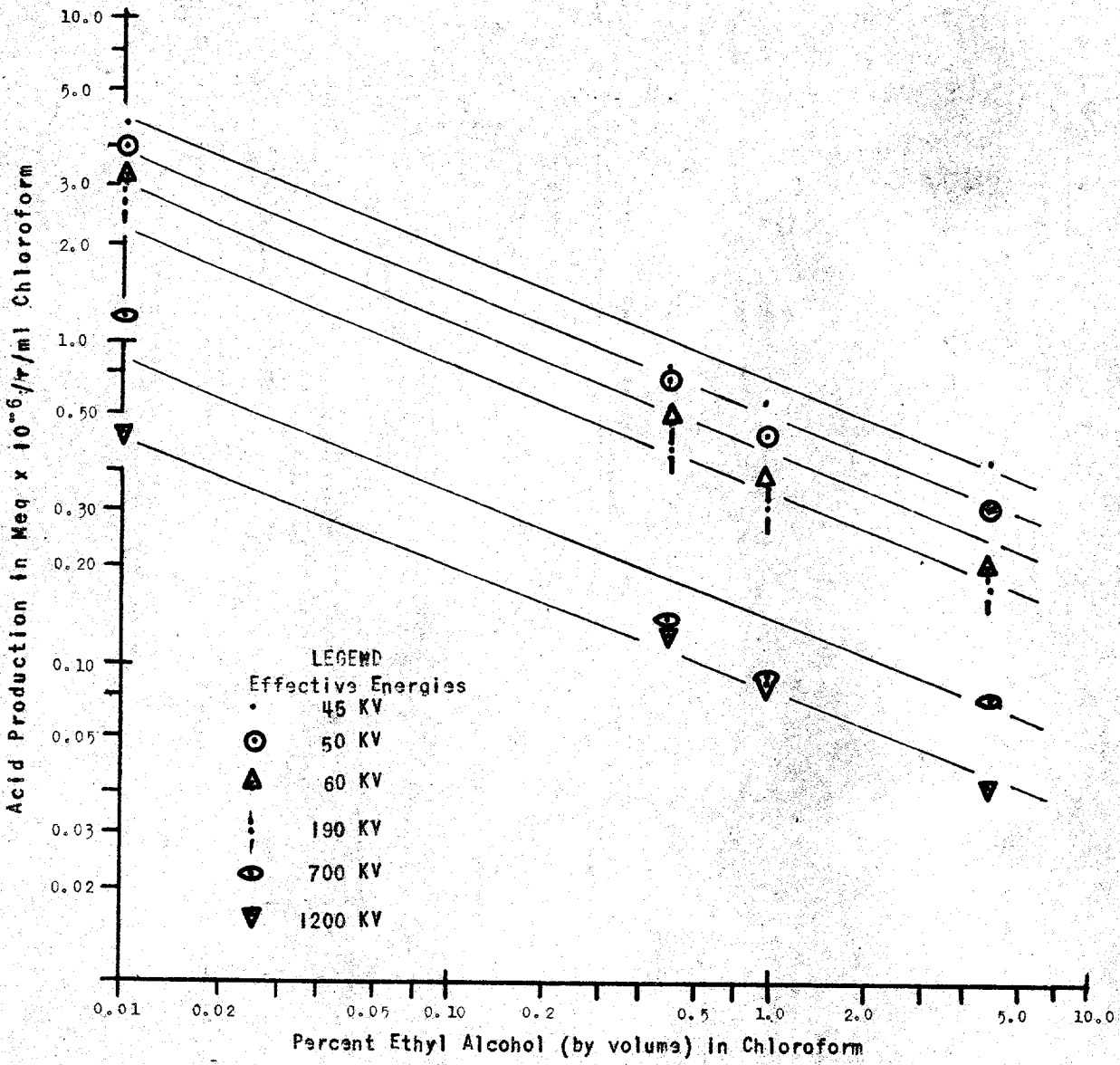
PREVENTION OF ACID EVOLUTION FROM CHLOROFORM
AFTER IRRADIATION BY THE ADDITION OF ETHYL ALCOHOL



Notes:
1. All samples radiated with 300 r (radium source)
2. Each point represents the mean of three separate determinations.
3. Each sample contained 1.0 ml chloroform and 0.5 ml dye.

Fig. 8

THE UNIFORM RELATION BETWEEN ACID PRODUCTION FROM CHLOROFORM AND ITS ALCOHOL CONTENT WHEN IRRADIATED WITH BEAMS OF DIFFERENT EFFECTIVE ENERGIES



Notes:

1. All samples radiated in quadruplicate in 2 ml pyrex glass stoppered volumetric flasks.
2. Each sample contained 1 ml chloroform and 0.5 ml dye.
3. Radiation Sources
   a) 85 KV Picker Portable Field X-Ray Unit
   b) 250 KV Picker Industrial X-Ray Unit
   c) 300 mgm Standardized Radium Source
   d) 96.4 curie Co^{60} Source
4. Effective Energies from sources described in (3)
   a) 40 KV
   b) 45, 50, 60, and 190 KV
   c) 700 KV
   d) 1200 KV

Fig. 9

apparent that a uniform relation exists between acid production from chloroform and the concentration of ethyl alcohol used to stabilize it, regardless of the effective energy of the radiation beam. This relation has theoretical significance regarding the mechanism of the chain reaction involved and its control by the action of alcohol in trapping but not destroying free radicals formed during the radiation-induced decomposition of chloroform.

COMMENT

As a result of the rate studies using the large Co^{60} source, it appears that when the chloroform-alcohol-dye system is irradiated at intensities exceeding 600-800 r/min, the acid evolution from chloroform preparations, containing various concentrations of alcohol stabilizer, becomes relatively constant. For practical purposes, this rate phenomenon means that small doses of *initial* bomb gamma radiation may be detected (with chloroform containing low percentages of alcohol) without using a large rate correction factor. However, to produce dosimeters capable of measuring either initial intense gamma radiation or slow contaminating bomb gamma rays, sufficient alcohol must be used in the system (2-2%) to resolve rate dependency throughout the broad ranges of radiation intensity likely to be encountered.

DIVERSIFIED PROBLEMS DIVISION - Code 91800

Stafford L. Warren, M.D.
Chief of Division

ALAMOGORDO SECTION - Code 91810

Kermit H. Larson
Chief of Section

Project #50015 - Radiation Induced Changes in Germinal and Somatic Mutation Rates of Selected Plants and Animals

Marion L. Queal, Ph.D., Albert W. Bellamy, Ph.D.

Two terminal reports dealing with exceptional females in *Platypoecilus maculatus* are in progress. They are to be completed during the next quarter. The tentative titles are:

1. Exceptional Females in *Platypoecilus maculatus* Crossing over in *P. maculatus.*
2. Crossing over and Atypical Breeding Behavior in *Platypoecilus maculatus.*

This then will terminate this project, as well as the Unit.

Project #50023 - The Study of the Fall-out of Fission Products and Other Bomb Products on Plants from the Alamogordo Area

K. H. Larson, W. F. Dunn, S. H. Gordon, J. H. Olafson, B. Gillooly

A summary of data obtained during the past years on plutonium content of plants from the Trinity Area, Alamogordo, New Mexico is ready for publication as a U.C.L.A. report. This report will conclude this project.

Project #50025 - Soil Studies with Respect to Radioactive Contamination in Fall-out Areas of Continental Testing Sites

K. H. Larson, J. H. Olafson, W. F. Dunn, H. M. Mork, J. W. Neel

Laboratory studies on soil samples collected from the Nevada Testing Site following last November's Test Detonations are in progress. The specific problems investigated have included particle size studies of the Fall-out materials at various locations, gross beta-gamma radioactivity, ratio of beta-gamma to alpha activity, gross alpha activity, and the solubility of the Fall-out materials in various solvents. Also in progress are greenhouse studies on the uptake of radioactive Fall-out matdrial from soil flats contaminated by actual Fall-out from the Sub-Surface Detonation. Studies are in progress on the rate of leaching of the surface contaminating Fall-out materials in the soil by irrigation water. A preliminary experiment has been conducted on feeding finely ground fused material to rats.

From the data accumulated to date the following generalized statements can be made:

1. The major portion of Fall-out particles from each Test Detonation in any one sample falls within a relatively narrow particle size range.

2. Significant amounts of the Fall-out materials are soluble in dilute acids with smaller amounts soluble in water.

3. A significant amount of the Fall-out materials on the soil flats is available to radishes, even in the first crop. Some indication of selective uptake of fission products is found in decay curves on plant material compared to the curves for decay of the total contaminating materials.

4. Evidence of leaching of the Fall-out materials has been found in the soil flat studies and in the soil profiles taken near the Crater areas in Nevada.

5. A significant portion of the radioactive fission products in soil and in the fused material collected from the Crater Area is absorbed from the G. I. tract of rats and principally retained in the carcass.

A preliminary report (Report UCLA-182) dealing with field observations at the time of the November Tests and preliminary laboratory data related to the Fall-out materials has been issued with a selected and limited distribution.

Project #50026 - Metabolism of Radioactive Plant Material in Mammals

W. J. Gross, J. Taylor, J. A. Lee

Inveetigations have been continued on the uptake and metabolism in rats of orally administered Sr^{90} + Y^{90} solution and aqueous suspension of plant dry material grown on Sr^{90} + Y^{90} contaminated soil.

It was previously reported that retention of total dosage by rats in a seven day feeding experiment was about eight percent for the Sr^{90} + Y^{90} solution group and about five percent for the barley feed group. Radio-assays two months after the first assay on five samples of femur ash demonstrated increased activity, indicating that Sr^{90} had probably been selectively deposited; the daughter produce Y^{90} approaching equilibrium with the selectively absorbed Sr^{90} in the later radio-assay causing the increase in activity.

To confirm the evidence pointing to selective uptake of Sr^{90} from a Sr^{90} + Y^{90} equilibrium mixture, three rats were given massive oral doses of Y^{91} in aqueous solution. When the fecal activity of these animals became low the animals were sacrificed and their tissue ash assayed radiologically. Results demonstrate in all three cases that less than 0.5 percent of the total dose was retained in the animal, therefore confirming the above observation that Sr^{90} must be assimilated and metabolized selectively from a Sr^{90} + Y^{90} equilibrium mixture.

Further experiments concerning the uptake and metabolism of orally administered Sr^{90} + Y^{90} in solution and in aqueous suspension of contaminated barley, consisting of daily, low activity doses, have been initiated and are to extend throughout a thirty day feeding period.

Project #50029 - The Uptake of Various Fission Products by Various Cultivated Crops from Soil

J. H. Olafson, J. W. Neel, S. H. Gordon, W. F. Dunn, B. Gillooly, H. M. Mork, A. J. Steen, K. H. Larson

Work is in progress on contaminating two soils (Hanford and Vina) with 100 dis/sec/gm of soil of several radioisotopes (Cs^{137}, Ce^{144} + Pr^{144}, Sr^{90} + Y^{90}, Y^{91}, Ru^{106} + Rh^{106}, Pm^{147} and Pu^{239}). Five crops (barley, beans, carrots, lettuce and radish) are to be planted in these soils by April 1, 1952 and will be grown to maturity and assayed for radioactivity. Later, tomatoes and alfalfa will be planted for a similar study.

A report is being prepared for publication on the previous work on the uptake of Cs^{137} and Sr^{90} + Y^{90} by various crops from Sorrento and Yolo soils.

Project #50030 - The Fixation of Several Isotopes and their Exchange from Various Soils and Clays in Aqueous Solution and Mammalian Body Fluids

K. H. Larson, J. W. Neel

There has been no work done on this project during the past quarter.

Project #50031 - A Study of the Uptake of some Fission Products by Barley from Various Soil Types

J. W. Neel, J. H. Olafsin, S. H. Gordon, B. Gillooly, W. F. Dunn, H. M. Mork, A. J. Steen, K. H. Larson

The study on the effect of varying amounts of Cesium carrier on the uptake of Cs^{137} from Hanford soil reported in the last Quarterly Report (Report UCLA-175) is being continued. Cesium nitrate (as carrier) was added at levels of five, ten, twenty, forty and eighty milligrams of Cs^+ per 100 grams of soil containing approximately 100 dis/sec/gm of soil of Cs^{137}. Further data have been obtained which indicate increasing uptake of Cs^{137} with increased amounts of carrier up to twenty milligrams of Cs^+ per 100 grams of soil. The relationship between carrier concentration used and the uptake of Cs^{137} is not linear at carrier concentrations which are not toxic to barley. No growth took place at the forty and eighty milligram levels of carrier due to toxicity. The following data are typical:

Mgms. Cs^+ Carrier per 100 gms Soil	Dis/sec/gm Plant Dry Material of Cs^{137}
5	276.
10	374.
20	422.

These data, when treated in such a way as to reflect the total uptake of Cs^+ show that between five to twenty milligrams Cs^+ per 100 grams soil

uptake is a linear function of concentration. They further indicate that a threshold value for greatly increased uptake lies somewhere between 0-5 milligrams Cesium carrier per 100 grams soil. Further work is planned to establish this threshold value.

Further work is in progress on the effect of varying amounts of Sr^{90} + Y^{90} fixed on soil on the uptake of the isotopes by barley. As previously reported, there is increased uptake with increasing activity levels from 0-3000 dis/sec/gm of soil but the relationship is not linear. The effect of each increment of additional activity becomes less as the upper limits under investigation are approached.

A report on the uptake of Cs^{137} and Sr^{90} + Y^{90} by barley from five soils is being prepared for publication.

MEDICAL PHYSICS SECTION - Code 91830

Benedict Cassen, Ph.D,
Chief of Section

Project #50001 - Study of the Physiological Effects of Air Blast (Old Title - Development of Blast Assay of Capillary Fragility)

Benedict Cassen, Ph.D., Katherine Kistler, Wanda Mankiewicz, Herbert Gass; Consultant - T. J. Haley, Ph.D.

Considerable confirmation was obtained to show that premedication of mice with mecholyl subcutaneously with 20 mg per kg and subdurally with 0.2 mg appreciably and significantly reduced the edematous lung weight of mice subjected dorsally to sixteen pounds shock pressure air blast while mounted on a rigid plate. Similar results were obtained with carcholin (another substituted choline) administered intraperitoneally with 1 mg per kg. No significant effect was obtained with any of the following drugs at dosages indicated.

Drugs	Dosage	Administered
Pilocarpine	50 mg per kg	Subcutaneous
Prostigmine	0.0025 mg	Subdural
	0.00025 mg	Subdural
	0.000025 mg	Subdural
	0.1 mg per kg	Intraperitoneally
Etamon Chloride	5 mg per kg	Intraperitoneally
Tridione	12.0 mg per kg	Intraperitoneally
Acetylcholine Chloride	0.5 mg	Subdural
Benedaine	5 mg per kg	Intraperitoneally
	10 mg per kg	Intraperitoneally
	20 mg per kg	Intraperitoneally
Hydergine	0.5 mg per kg	Intraperitoneally
	0.1 mg per kg	Intraperitoneally
	0.01 mg per kg	Intraperitoneally

This survey will be continued with several other types of drugs.

A series of tests were made by administering mecholyl subdurally immediately after blasting. The lung weights in these post-medicated animals did not differ significantly from the blasted controls.

A Horsley-Clark stereotaxic locator was received. Some features of it had to be rebuilt to properly adapt it to rat brain localization. With the kind assistance of Prof. H. Magoun and Dr. Earl Eldred of the Department of Anatomy of the Medical School a lesion localizing procedure and calibration was worked out. As first test of the procedure a verification was made of the effects reported by Patton and Gamble, that specific hypothalamic lesions would produce lung edema in rats. In proportion, the lung edema produced by these lesions was not nearly as drastic as the quickly fatal lung edema produced in mice by air blast. The relation, if any, of these lung edema effects is still very obscure.

A method was developed for mounting a micro-injection device on the stereotaxic locator. Attempts will be made to produce lung edema by the micro-injection of various drugs at various hypothalamic sites. Work in this direction is now in progress.

Project #50003 - Development of Photomultiplier Radiation Detecting Devices

Benedict Cassen, Ph.D., Lawrence Curtis

A series of sensitivity and background counts were obtained on four calcium tungstate scintillation counters with and without heavy lead shielding. The counts were plotted against plate voltage on semilogarithmic paper. In the voltage regions where the semilogarithmic slope was low it was found that heavy lead shielding eliminated up to eighty percent of the background. This degree of shielding is impractical for uptake measurements on patients but is very desirable for laboratory gamma counting of whole tissue pieces or other biological specimens.

Project #50033 - Development and Testing of Automatic Scanning Gamma Locator

Benedict Cassen, Ph.D., Lawrence Curtis; with the aid of Franz Bauer, M.D. and William Goodwin, M.D. of the Sawtelle Veterans Administration Hospital

Well over one hundred thyroid patients have been given a sufficient dosage of I^{131} to enable a visualizable record of the active parts of their thyroid glands to be obtained. These pictures are now called "scintigrams". The success of this device in a big hospital has been sufficiently great that several other big hospitals have purchased similar equipment from a commercial source and many more seem to be interested.

A method for speeding up and improving the contrast of these thyroid scintigrams was developed on the Project. A ratemeter circuit attached to the output of the scaler controls a relay so that if the rate is below a certain preset value, the printer circuit is opened and the scanning motor goes faster than normal. The net effect is shown in Figs. 10b and 10c of this section's report. Fig. 10b shows the conventional scintigram of an enlarged hyperthyroid gland. Fig. 10c shows the effect on the same patient of increasing the visual contrast and speeding up the background scanning.

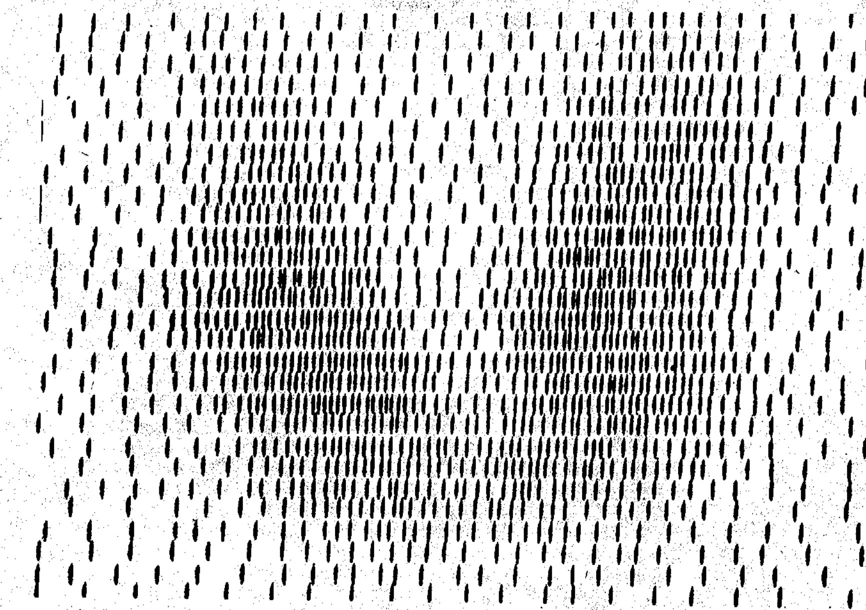
Project #50035 - Spark Shadowgraph

Benedict Cassen, Ph.D., Brian B. Dunne, Jr., Herbert Gass

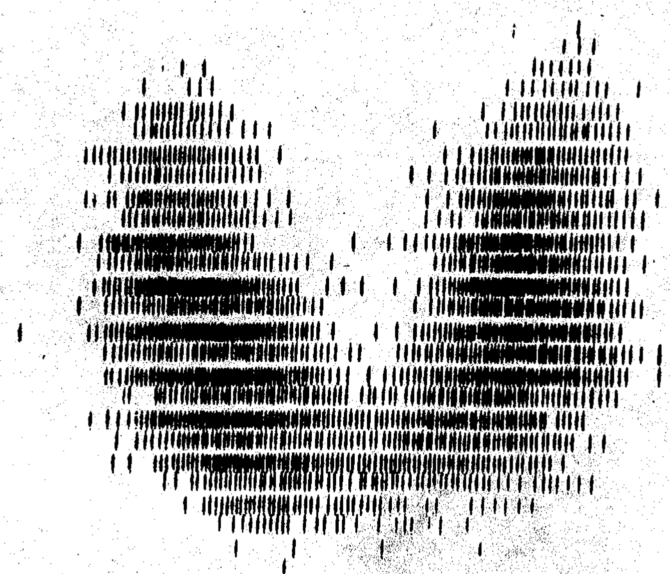
In this period a spark shadowgraph has been constructed and put into operation. So far, only one spark unit is being tested. On preliminary tests beautiful pictures of the refraction of shock waves going over obstacles were obtained. The reflection pattern of shock waves from the body of a mouse could be photographed. This effect is shown in Fig. 10a of this section's report. Also some preliminary pictures of the high speed jets from "Hypospray" jet injectors were obtained.

An order was placed for a new type of relatively inexpensive x-ray microflash equipment operating from sixty to one hundred kv. The use of this equipment will enable x-ray pictures of the skeleton of a small animal to be obtained with an exposure of less than one millionth of a second.

A



B



C

A. Spark shadowgraph of shock waves being reflected from the body of a mouse. The incident shock wave is travelling from right to left and can be seen over the head of the vertically mounted animal.

B. Thyroid scintigram of diffuse hyperthyroid patient made by the regular method.

C. Improvement of contrast of thyroid scintigram produced by new method.

Fig. 10