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Health and Biology

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QUARTERLY TECHNICAL REPORT

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Submitted by: Henry A. Blair
Director

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Health and Biology

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INTRODUCTION

The scientific work presented herein has been coded at the program and problem levels according to the scheme given on Pages 7 and 8. In the report all contributions to a given problem have been assembled together without regard to the administrative organization except that the number of the section which did the work is prefixed in each case. By using this number, it can be found on Page 12 what administrative officer can be approached for information about particular work.

It should be noted that the Quarterly Technical Reports of The University of Rochester Atomic Energy Project do not attempt to describe progress in all of the research programs but only in those in which some significant results have been achieved but which are not sufficiently complete to be written up as a final report.

EXPLANATION OF PROGRAM AND PROBLEM CODES

The scientific work at The University of Rochester Atomic Energy Project has been coded at the program and problem levels. The programs, in general, indicate broad fields of investigative or service activities while the problems indicate divisions of these fields. Although no consistent method of division in problems was possible, an attempt was made to achieve a natural division in the sense that each problem would encompass a subject normally written up and generally considered as a unit. The program on chemical toxicity of uranium, for example, has been broken down into problems according to the divisions commonly employed by toxicologists.

The problem codes are not related directly to the administrative organization of the Project. Consequently, the smallest administrative unit, the section, may work on more than one of the coded problems. Conversely, more than one section may work on the same coded problem. The administrative organization will be ignored in making this quarterly report of our research and service activities, all material being assembled according to the program and problem codes. The contribution of each section to a Quarterly Technical Report will be prefixed by the section number, however, to permit reference to the administrative organization if necessary.

It has not been possible to code the problems sufficiently broadly to avoid all overlapping. In cases in which various parts of a given investigation might be coded differently, the whole work was coded according to its principal subject matter as long as the minor subjects were relatively unimportant. Otherwise, the work was divided under appropriate codes.

PROGRAM AND PROBLEM CODES

I. X.R. BIOLOGICAL EFFECTS OF EXTERNAL RADIATION (X-RAYS AND γ RAYS)

- X.R.1 Tolerance Studies (dose levels, survival time, gross and histo-pathology)
- X.R.2 Mechanism of Effects (physiological and biochemical)
- X.R.3 Therapy (measures against radiation effects)
- X.R.4 Hematology
- X.R.5 Genetics (histogenetics)
- X.R.6 Embryology
- X.R.7 Bacteriology and Immunology

II. I.R. BIOLOGICAL EFFECTS OF EXTERNAL RADIATION (INFRA-RED & ULTRA-VIOLET)

- I.R.1 Flash Burns

III. R.M. BIOLOGICAL EFFECTS OF RADIOACTIVE MATERIALS (CONTACT, INGESTION, ETC.)

- R.M.1 Polonium
- R.M.2 Radon
- R.M.3 Thoron
- R.M.4 Miscellaneous Project Metals

IV. U. URANIUM

- U.1 Physical and Chemical Properties
- U.2 Toxic Effects (description of acute and chronic toxicity)
- U.3 Toxic Limits (respiratory; oral; skin; eye; parenteral)
- U.4 Fate (distribution and excretion)
- U.5 Mechanism of Toxic Effects
- U.6 Methods of Detection of Poisoning, Prophylaxis, Treatment and Protection

V. Be. BERYLLIUM

Be.1 Physical and Chemical Properties

Be.2 Toxic Effects (description of acute and chronic toxicity)

Be.3 Toxic Limits (respiratory; oral; skin; eye; parenteral)

Be.4 Fate (distribution and excretion)

Be.5 Mechanism of Toxic Effects

Be.6 Methods of Detection of Poisoning, Prophylaxis, Treatment and Protection

VI. Th. THORIUM

Th.1 Physical and Chemical Properties

Th.2 Toxic Effects (description of acute and chronic toxicity)

Th.3 Toxic Limits (respiratory; oral; skin; eye; parenteral)

Th.4 Fate (distribution and excretion)

Th.5 Mechanism of Toxic Effects

Th.6 Methods of Detection of Poisoning, Prophylaxis, Treatment and Protection

VII. F. FLUORIDE

F.1 Physical and Chemical Properties

F.2 Toxic Effects (description of acute and chronic toxicity)

F.3 Toxic Limits (respiratory; oral; skin; eye; parenteral)

F.4 Fate (distribution and excretion)

F.5 Mechanism of Toxic Effect

F.6 Methods of Detection of Poisoning, Prophylaxis, Treatment and Protection

VIII. S.M. SPECIAL MATERIALS

S.M.1 Physical and Chemical Properties

S.M.2 Toxic Effects (description of acute and chronic toxicity)

S.M.3 Toxic Limits (respiratory; oral; skin; eye; parenteral)

S.M.4 Fate (distribution and excretion)

S.M.5 Mechanism of Toxic Effect

S.M.6 Methods of Detection of Poisoning, Prophylaxis, Treatment and Protection

IX. I.S. ISOTOPES

I.S.1 Tracer Chemistry

I.S.2 Radioautography

I.S.3 Therapy

X. O.S. OUTSIDE SERVICES

XI. P.H. PROJECT HEALTH

XII. H.P. HEALTH PHYSICS

H.P.1 Research and Development

H.P.2 Service

XIII. C.S. SPECIAL CLINICAL SERVICE

XIV. I.N. INSTRUMENTATION (SPECTROSCOPY, ELECTRON MICROSCOPY, X-RAY AND NUCLEAR RADIATION DETECTORS, X-RAY DIFFRACTION, ELECTRONICS)

I.N.1 Research and Development

I.N.2 Service

I.N.3 Instrumentation for Outside Organizations

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3130	Radiation Tolerance	John B. Hursh
3136	Radiation Physiology	John B. Hursh
3140	Radiation Chemistry	Kurt Salomon
3150	Spectroscopy	Luville T. Steadman
3160	Radiation Mechanics	Michael Watson
3170	Radiation Toxicology	J. Newell Stannard
3171	Radioautography	J. Newell Stannard

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UNCLASSIFIED

PROGRAM X.R.

BIOLOGICAL EFFECTS OF EXTERNAL RADIATION (X-RAYS AND γ RAYS)

Problem Code: X.R.2 (Mechanism of Effects)

Section Code: 3120

Authors: L. L. Miller and Mrs. E. Gates

The Effect of Whole Body Radiation on Plasma Peptidase Activity and Plasma Fibrinogen Content.

Possible Indicators of Injury From Low Dosage Whole Body Radiation.

Background: The demonstrable biological effect in the dog of low dosages of total body x-radiation of the order of 25-50 r have been limited primarily to the temporary decrease (if any) in the circulating white cell count. It has also been known; 1. That at certain dosage levels there is, at some time, an increase above normal of cellular division which is preceded or followed by a decrease in the mitotic process (1); 2. That the total bone marrow and lymph nodes of mammals have a large reserve capacity for the production and release of granulocytes and lymphocytes respectively; 3. That the granulocytes (2) and lymphocytes (2) contain a variety of proteolytic and peptide splitting enzymes. Whether these enzymes represent the sole precursor or precursors of the plasma protease and peptidase activity under normal conditions is not known.

On the basis of the above known facts, it seems reasonable that a small dose of x-radiation could damage circulating leukocytes causing them to disintegrate prematurely and could first accelerate and then depress the production and release of leukocytes. If the proteases and peptidases of disintegrating leukocytes are discharged into the circulating plasma, one may conceive of a situation in which a greater than normal amount of peptidase is present in the serum or plasma without

the leukocyte count being significantly reduced. The detection of increased serum or plasma protease or peptidase activity under such circumstances may afford a simple test for detecting low dosage x-radiation injury.

In addition it is known that the liver responds to minimal tissue damage in other organs or tissues, or in itself with an increased production and release of fibrinogen; this is manifest with any acute inflammatory process and has been seen under conditions where the liver is affected by such small amounts of hepatoxic agents as will not produce definite histologic evidence of tissue damage. Here again it seemed reasonable to expect some elevation of plasma fibrinogen to follow even low dosage whole body radiation.

Method: The animals were healthy, normal, adult dogs maintained on an adequate ration of Purina Dog Chow.

After a suitable preliminary period of sham radiation the dogs were given radiation according to the details of the individual protocols presented below.

Blood samples were drawn from the external jugular veins after the animals had been without food for 14 to 18 hours. Samples were taken during preliminary control periods and at intervals up to 20 days after radiation.

The rate of hydrolysis of the substrate LA, (leucineamide) and BAA, (benzoylargininеamide) by dog sera 1380, 1381, 1398 and 1399 was followed by means of the Grassman-Heyde titration method for amino acids. The action of the remaining sera upon the substrates was studied by measurement of NH_3 liberated from the amide LA and BAA as outlined by Van Slyke (for the liberation of ammonia after cerease action).

The test solutions in all cases contained either:

(1) Leucineamide hydrochloride ═ .05 mM per ml of test solution, $MnSO_4$ ═ 1×10^{-3}, final concentration; Veronal buffer .1M of

pH 7.8 - 8.0; fresh serum .4 ml serum per ml test solution.

(2) Benzoylarginineamide .HCl═══.05 mM per ml test solution; cysteine HCl═══1×10^{-2}M, final concentration; citrate buffer .1M of pH c.0 - 5.2; fresh serum═══.4 ml serum per ml test solution.

In all runs the buffered serum was allowed to incubate with the activator for 1 hour prior to the addition of substrate.

The fibrinogen and plasma protein content of the sera was determined by the micro Kjeldahl method using mercury and copper catalysts, distillation, and titration.

Separation of the globulin fraction of control normal serum was attempted by means of: 1. alcohol and 2. dilute acetic acid. The globulin fraction was dissolved in buffer and activation was attempted with freshly distilled $CHCl_3$ and streptokinase. In no case did the treatment produce a significant increase in the rate of hydrolyses of the substrates LA or BAA over that of untreated dog serum.

Results: Table 1 (Page 16) shows the changes noted in serum peptidase activity after single doses of whole body radiation of 40 r or 200 r. There are definite large increases in serum peptidase activity noted in the 24 hour specimens. In the case of both dosage levels the serum peptidase activity is restored to normal control levels at 10 days after radiation.

Table 2 (Page 17) indicates that the increase in serum LA peptidase activity at single dosage levels of 250 r or 430 r are small or insignificant. If anything, there appears to be a transient decline in BAA peptidase activity at 24 hours followed by a slight apparent increase and a fall almost to zero activity at 10 days.

Table 3 (Page 18) shows the results of a single total body radiation dose of 300 r given over 24 hours. Here again the effect on LA or BAA peptidase activity is insignificant. However, the plasma fibrinogen shows a steady rise

TABLE 1

Dog No. Radiation Dose		Control	24 Hours After Radiation	48 Hours After Radiation	7 Days After Radiation	10 Days After Radiation
Dog 1380 40 r at rate of 150 r/hr	LA	16.3	15.9	16.7	19.9	4.1
	BAA	6.4	32.5	13.1	11.1	9.9
Dog 1381 40 r at rate of 150 r/hr	LA	12.1	16.1	15.4	18.1	8.8
	BAA	5.9	21.2	13.8	11.7	4.8
Dog 1398 200 r at rate of 150 r/hr	LA	13.0	24.1	7.0	21.6	10.5
	BAA	3.3	18.2	15.0	13.0	1.7
Dog 1399 200 r at rate of 150 r/hr	LA	16.1	37.1	9.1	24.7	11.3
	BAA	3.7	19.3	11.7	24.4	4.1

Dogs given varying doses of radiation at the same rate, namely 150 r/hr.

Hydrolysis of substrates here measured by liberation of amino acids from the amides LA and BAA as titrated by Grassman-Heyde method. The figures represent the % hydrolysis after 3 hours incubation at 39°C.

The radiation for all experiments produced by a one million volt peak G.E. X-Ray generator with an aluminum parabolic filter. (Half value layer of this source is equal to 5.12 mm lead). Target to animal center distance equalled 68 inches).

TABLE 2

Dog No. Radiation Dose		Control	24 Hours After Radiation	48 Hours After Radiation	7 Days After Radiation	10 Days After Radiation
Dog 1445 250 r at rate of 150 r/hr	LA	2.6	5.4	7.1	4.8	1.6
	BAA	2.7	0.7	2.5	1.4	0.3
Dog 1452 250 r at rate of 150 r/hr	LA	8.4	8.0	6.6	6.8	9.3
	BAA	2.0	0.4	2.3	2.1	0.5
Dog 1484 430 r at rate of 150 r/hr	LA	7.6	13.3	5.8	4.9	7.9
	BAA	2.4	1.1	0.7	2.0	0.3
Dog 1487 430 r at rate of 150 r/hr	LA	7.6	5.5	4.8	6.0	6.2
	BAA	4.2	2.6	1.9	2.2	0.8

Dogs given 250 r or 430 r doses of radiation at constant rate of 150 r/hr.

Peptidase activity here measured by liberation of NH_3 from amides LA and BAA.

The figures represent the % hydrolysis after 3 hours incubation at 39°C.

TABLE 3

Dog 1435 Dose 300 r	Fibrinogen (As Fibrin	Total Plasma Protein	% Hydrolysis of LA After 3 hr at 39.5°C	% Hydrolysis of BAA After 3 hr at 39°C
Control	302 mg %	6.81%	8.0	1.6
Control	300	6.76	9.6	2.0
24 hr after end of radiation	334	6.34	10.0	2.0
48 hr after end of radiation	361	6.95	9.9	2.1
6 days after end of radiation	362	6.84	8.9	1.9
10 days after end of radiation	378	7.07	9.3	2.0
15 days after end of radiation	394	No plasma available	6.4	.8
20 days after end of radiation	319	6.71	6.8	2.0
Dog 1478 Dose 300 r				
Control	290 mg %	7.64%	4.0	2.0
Control	290	7.59	5.6	1.8
24 hr after end of radiation	329	7.44	7.2	2.8
48 hr after end of radiation	358	6.95	8.8	4.0
6 days after end of radiation	364	6.25	5.7	3.5
10 days after end of radiation	373	6.80	7.3	2.9
15 days after end of radiation	349	No plasma available	8.9	3.4
20 days after end of radiation	304	6.65	8.0	3.6

Dogs 1435 and 1478 - dose 300 r over a period of 24 hours at a rate of 12.5 r/hr.
Serum and plasma used same day it was drawn with exception of plasma protein determinatio

to a maximum at 10-15 days after the end of radiation. This corresponds to the time peak of the clinical disturbance, if any.

Table 4 (Page 20) shows fibrinogen and LA and BAA peptidase activity after 1000 r of whole body radiation was given at a continuous rate of 41.5 r per hour for 24 hours. The only outstanding effect on the peptidase activity is the decline in the BAA peptidase virtually to zero level. This decline is apparently most pronounced in the last three days of life. Similarly, during the last three days of life the plasma fibrinogen rises to levels 3 to 4 times as high as the normal control levels.

Discussion: The implications of these preliminary experiments are not entirely clear. However, especially at the low dosage levels of 25 to 50 r, the serum peptidase activity changes warrant further study especially in the first 24 hours after radiation. In addition it is intended to make observations on any changes in plasma fibrinogen which may occur.

If the serum peptidase activity consistently shows increases after low dosage whole body radiation, this biochemical change may well represent a measurable factor for assessing the extent of tissue injury.

At the high dosage levels it is conceivable that measurement of BAA peptidase activity and plasma fibrinogen may afford prognostic criteria, especially for following the value of therapeutic agents in the treatment of radiation injury.

Bibliography

1. Knowlton, N. P., Jr., Hempelman, L. H., and Hoffman, J. G., Science, 107, 625 (1948)

Hoffman, R. S. and Wollman, S. H., Proc. Soc. Exp. Biol., 70, 38-40 (1949)

2. Opie, E. L., J. Exp. Med., 7, 316 (1905)

Husfeldt, E., J. of Physiol. Chem., 194, 137 (1931)

TABLE 4

Dog 1492 Dose 1000 r	Fibrinogen (As Fibrin)	Total Plasma Protein	% Hydrolysis of LA After 3 hr at 39.5°C	% Hydrolysis of BAA After 3 hr at 39.5°C
Control	233 mg %	6.72%	9.6	2.2
Control	234	6.73	8.8	2.3
24 hr after end of radiation	294	6.48	11.3	2.7
48 hr after end of radiation	285	6.51	3.1	1.0
72 hr after end of radiation	*	6.54	5.8	0.9
6 days after end of radiation	476**	6.37	9.3	2.2
7 days after end of radiation	925	6.74	6.9	1.0
9 days after end of radiation	1152	7.12	2.4	0.0
Dog 1515 Dose 1000 r				
Control	300 mg %	6.48%	8.9	2.3
Control	305	6.47	8.7	1.7
24 hr after end of radiation	353	7.58	6.4	2.9
48 hr after end of radiation	374	6.30	9.0	0.5
72 hr after end of radiation	*	9.43	6.8	0.5
6 days after end of radiation	1050**	6.71	8.1	0.4

*Fibrin clot formation incomplete.

**Fibrin clot formation incomplete until beef thrombin and rabbit brain thromboplastin were added

Dogs 1492 and 1515 were given a dose of 1000 r over a period of 24 hr at a rate of 41.6 r/hr. 1492 died at the end of 9 days - 1515 died at the end of 6 days

Problem Code: X.R.2 (Mechanism of Effects)

Section Code: 3140

Author: K. Salomon

Some Aspects of Glycine Metabolism in Bone Marrow Homogenates

Background: It has recently been demonstrated that the α-carbon atom of glycine participates (1) in the synthesis of bone marrow fats. This observation may best be explained by presuming that glycine is converted to acetate which has been shown to be the primary precursor of body fats (2). Recent reports tend to support this view. Thus, Sprinson (3) has shown that rats fed $C^{14}H_2NH_2COOH$ in conjunction with 1-α-amino-γ-phenyl butyric acid excrete acetyl-1-aminophenyl butyric acid and that the acetylated amine contains equal C^{14} activity in both carbon atoms of the acetyl residue. It must be concluded from this experiment that the α-carbon atom of glycine contributes to both carbon atoms of acetate. In order to ascertain whether the over-all reaction:

$$CH_2NH_2COOH \longrightarrow CH_3COOH$$

could be reversed, experiments were set up with rabbit bone marrow homogenates containing $C^{14}H_3COOH$ to bring forth direct evidence bearing on this point. While this work was in progress, a report (4) appeared indicating that the feeding of labeled acetate resulted in the incorporation of low, but significant C^{14} activity in hippuric acid isolated from the urine of rats so fed. It appears from these data that the methyl carbon atom of acetate contributes to both α- and carboxyl carbon atoms of glycine, whereas the carboxyl carbon atom of acetate contributes only to the carboxyl carbon atom of glycine. Experiments to be described in this report indicate that rabbit bone marrow homogenates are capable of converting acetate to glycine, a fact with which one must reckon in

the study of the mechanism of hemin synthesis.

The second portion of this report is concerned with a new observation concerning glycine metabolism. In general, two possible pathways of glycine metabolism are now being seriously considered. Firstly, glycine may be converted to glyoxylic acid which, in turn, condenses with another molecule of glycine to form a 4-carbon compound as a precursor of oxalacetic acid (5,6). Secondly, glycine might be converted to formic acid which then condenses with another molecule of glycine to give rise to serine, pyruvate, etc. (7,8,9). The data to be presented here cannot be adequately explained on the basis of either scheme necessitating the postulation of a new scheme.

Methods: Complete bone marrow homogenates were prepared and additions of acetate and glycine were made, essentially as described previously (1). In all cases the incubation period was three hours at 38°C. At the end of this period, the incubated material (total volume approximately 30 ml.) was high-speed centrifuged, resulting in separation of fats (at top of tube), insoluble particles (at bottom) and straw-colored liquid. This liquid was withdrawn and glycine isolated from it. The carbon dioxide evolved during the incubation was collected in 10 N KOH in the side arm of a large Warburg vessel in which the experiment was conducted. Retention of carbon dioxide was eliminated by tipping into the center compartment of the vessel 2N H_2SO_4 before removal of the vessel from the manometer.

Before glycine was isolated, the liquid was steam distilled in order to remove any volatile acids. The residue after steam distillation was concentrated to a small volume (ca. 10 ml), adjusted to pH 5.5 with K $H_2PO_4Na_3PO_4$ buffer and then treated with 1% Ninhydrin in a stream of N_2 with gentle heating. The CO_2 representing the -COOH group of glycine was collected in alkali with continuous washing with N_2 and isolated as $BaCO_3$. After removal of CO_2 the solution was

distilled into a methanolic solution of dimethyl dihydroresorcinol so as to make a final methanol concentration of 50%. The resulting distillate was then refluxed gently with the dimethyl dihydroresorcinol for 10 minutes. The resulting derivative of formaldehyde, methylene bis-methone, was isolated in crystalline form (MP 189°C) and recyrstallized from water.

Results: The data presented in Table 1 below demonstrate in a direct manner that conversion of acetate to glycine takes place in rabbit bone marrow homogenates. Under the prevailing in-vitro conditions, which may not be optimal, approximately 2% of the α-carbon atome of acetate is incorporated into the α-carbon atom of glycine within three hours. It should be pointed out that the millimolar C^{14}-activities shown in column II have not been corrected for the known amount of inert glycine present during the experiment. This factor, however, has been taken into account in computing columns III and IV. The distribution of C^{14}-activity among the two carbon atoms of glycine has not been studied at this time.

TABLE 1

CONVERSION OF ACETATE TO GLYCINE

Expt. No.	I. Millimolar C^{14}-Activity of $C^{14}H_3COOH$ Added.	II. Millimolar C^{14}-Activity of α-Carbon Atom of Glycine.	III. % Acetate Converted to Glycine.	IV. μM Glycine Synthesized from Acetate.
1.	300	3.6	1.2	7
2.	300	5.1	1.7	10

All C^{14}-Activities are Expressed as 10^4 Disintegrations per min. per mM.

In Table 2 (Page 25) data pertaining to the metabolism of glycine in bone marrow homogenates are presented. Experiments 1 - 3 are comparable in all respects except that in experiment 2 the amount of glycine added was increased three-fold, together with a more than two-fold increase in millimolar C^{14}-activity. In experiment 3, on the other hand, the amount of inert acetate added was increased two-fold. In experiments 4 - 6, the amount of bone marrow homogenate added was varied from one-fourth of that used in experiments 1 - 3 (experiment 4), to one-half (experiment 5), and three-fourths (experiment 6).

The initial activity of the methylene-labeled glycine added is shown in column I of Table 2 (Page 25), whereas the final C^{14}-activity is listed in column II. By methods described earlier, glycine was degraded so that the C^{14}-activity of the two carbon atoms could be measured separately. This is set forth in columns IIa and IIb. The localization of C^{14}-activity in the glycine preparation added to the homogenate is unequivocal on the basis of the method of synthesis. It is, thus, apparent that appreciable dilution of the α-carbon atom of glycine has occurred within three hours, and, consequently, that additional glycine has been synthesized by the system either as a result of proteolysis or due to some other pathway(s). It is also apparent that the bulk of the newly formed glycine does not originate from acetate since in experiment 3 in which larger amounts of inert acetate were added no greater dilution of the C^{14}-activity of the α-carbon atom of glycine occurred. In general, the dilution of the α-carbon atom of glycine is of the order of 1.5 as shown in column III. The only exception presents itself in experiment 2 where an increased amount of glycine added to the system resulted in higher dilution of C^{14}-activity. The degree of dilution is somewhat greater than might have been expected and this may be due to a "sparking" effect of higher glycine concentrations resulting

TABLE 2

Expt.	I	II		III	IV	V	VI	VII
	Millimolar C^{14}-Activity of $C^{14}H_2NH_2COOH$ Added (C_o)	Millimolar C^{14}-Activity of Glycine (free) isolated at end of expt.		C_o/C_1	C_o/C_2	C_1/C_2	% of-COOH Carbon Atom of Glycine Derived from α-Carbon Atom	Millimolar C^{14} Activity of CO_2 Evolved During the Incubation Period
		a. α-Carbon Atom (C_1)	b. -COOH Carbon Atom (C_2)					
1	340	217		1.6				1.2
2	690	62	3.9	11.0	172	16	0.6	0.7
3	340	259	6.6	1.3	51	40	2.0	1.2
4	340	275		1.2				
5	340	181	6.0	1.9	57	30	1.8	
6	340	246	8.4	1.4	40	30	2.5	

All C^{14} Activities Are Expressed as 10^4 Disintegrations per Min. per mM.

in the formation of increased amounts of glycine by the system, analgous to "sparking" effects seen in the tricarboxylic acid cycle.

The data shown in Table 2 (Page 25), furthermore, indicate that the α-carbon atom of glycine also contributes to the carboxyl carbon atom of newly synthesized glycine, as is apparent from inspection of column IIb. When calculated on the basis of C_o, the dilution of activity is about 50-fold, viz. column IV. These calculations are probably not entirely adequate since they do not take into account the simultaneous dilution of the α-carbon atom of glycine taking place in the course of the experiment. Thus, the two limiting values for the dilution constant are shown in columns IV and V, respectively, column V representing the dilution on the basis of the highest dilution of the α-carbon atom of glycine. From column VI it is apparent that approximately 2% of the carboxyl carbon atom of glycine at the end of the experiment is derived from the α-carbon atom.

Another point in this experiment seems noteworthy, namely the presumptive source of the CO_2 evolved during the experiment. From data presented here, it would appear likely that the CO_2 is derived, to a considerable extent, from the carboxyl carbon atom of glycine since the activities are of an order compatible with such an assumption. It has been shown that formate is formed from the α-carbon atom of glycine (8) and 48% of formate (7), under <u>in-vivo</u> conditions, is converted to respiratory CO_2 within 14 hours. Since the C^{14}-activity of the

carbon atom of glycine in bone marrow homogenates. The mechanism operating in this conversion is as yet unclear, although it would seem probable that acetate would be converted to pyruvate via the tricarboxylic acid cycle and that pyruvate, in turn, would give rise to serine which is in biological equilibrium with glycine.

The experiments constituting the second part of this report indicate a metabolic relationship between the α-carbon atom and the carboxyl carbon atom of glycine in rabbit bone marrow homogenates. The finding that the α-carbon atom of glycine contributes to the carboxyl carbon atom of glycine forces one to postulate an alternate metabolic scheme for glycine, since the two schemes referred to earlier cannot adequately account for these observations. On the basis of the two prevailing theories, the α-carbon atom of glycine could not conceivably find its way to the carboxyl carbon atom. In order to explain the data presented here, it seems best to postulate a symmetrical compound "glyoxal" as the primary metabolic reaction product of glycine. Such a symmetrical product could then condense with equal probability at either end of the molecule with another molecule of glycine and thus give rise to two differently labeled 4-carbon compounds, one of which finally would give rise to glycine. The following hypothetical scheme is thus proposed:

The Postulated "Internal Dismutation" of Glycine

$$C^{14}H_2NH_2\text{-}COOH \longrightarrow \left[H\text{-}\underset{\underset{O}{\|}}{C^{14}}\text{-}\underset{\underset{O}{\|}}{C}\text{-}H\right] \longrightarrow \qquad (1)$$

Glyoxal

(Hypothetical Symmetrical Intermediate)

$$C^{14}H_2NH_2\text{-}COOH + \left[H\text{-}\underset{\underset{O}{\|}}{C^{14}}\text{-}\underset{\underset{O}{\|}}{C}\text{-}H\right] \longrightarrow HOOC\text{-}\underset{\underset{O}{\|}}{C^{14}}\text{-}C^{14}H_2\text{-}COOH\,(50\%) + HOOC^{14}\text{-}\underset{\underset{O}{\|}}{C}\text{-}C^{14}H_2\text{-}COOH\,(50\%) \qquad (2)$$

$$HOOC^{14}\text{-}\underset{\underset{O}{\|}}{C}\text{-}C^{14}H_2\text{-}COOH \longrightarrow HOOC^{14}\text{-}\underset{\underset{O}{\|}}{C}\text{-}C^{14}H_3 + CO_2 \qquad (3)$$

$$HOOC^{14}\text{-}\underset{\underset{O}{\|}}{C}\text{-}C^{14}H_3 \longrightarrow HOOC^{14}\text{-}\underset{\underset{NH_2}{|}}{C}\text{-}C^{14}H_2OH \longrightarrow HOOC^{14}\text{-}CH_2NH_2 + HC^{14}OOH \qquad (4)$$

It is apparent from the data that this proposed "internal dismutation" occurs only to a relatively limited extent, i.e. approximately 4%. It can not be stated at this time whether reactions (1) or (2) would be the limiting factor. The anticipated chemical instability of the hypothetical symmetrical compound is in accord with the limited extent of occurrence of this reaction.

The proposed hypothesis would also explain the low levels of C^{14}-activity found in the metabolic CO_2, which is thought to arise from the product of reaction (4) in the following way:

$$CH_2NH_2\text{-}C^{14}OOH \longrightarrow HCOOH + C^{14}O_2 + NH_3$$

It should be emphasized that the foregoing comments are entirely speculative and that further experimentation will be necessary before any definite conclusion as to the mechanism of the conversion of the α-carbon atom of glycine to the carboxyl carbon atom of glycine can be drawn. Further evidence for the formation of glyoxal will be obtained from an attempt at trapping this dialdehyde as the dioxime. Another possible approach to the study of the mechanism of this metabolic reaction would be the isolation of oxalacetic acid which, if this scheme is operating, should contain C^{14}-activity in the α and β carbon atoms as well as in the carboxyl carbon atom adjacent to the carboxyl group.

Summary:

1. It has been demonstrated that the methyl carbon atom of acetate is converted to the methylene carbon atom of glycine in bone marrow homogenates.

2. It has been shown that the α-carbon atom of glycine contributes to the carboxyl carbon atom of glycine when glycine is incubated with bone marrow homogenates.

3. The origin of metabolic CO_2 has been commented upon.

4. A hypothetical scheme explaining the results summarized in 2 and 3 has been presented.

Bibliography

1. Altman, K. I., J. Biol. Chem., 177, 985 (1949)

2. Rittenberg, D. and Bloch, K., ibid, 154, 311 (1944); 160, 417 (1945)

3. Sprinson, D. B., ibid, 178, 529 (1949)

4. Arnstein, H. R. V. and Neuberger, A., Biochem. J., 45, iii (1946)

5. Ratner, S., Nocito, V. and Green, D. F., J. Biol. Chem., 152, 119 (1944)

6. Barker, H. A., Volcani, B. E., and Cardon, B. P., ibid, 173, 803 (1948)

7. Sakami, W., ibid, 176, 995 (1948); 178, 529 (1948)

8. Siekevitz, P. and Greenberg, D. M., ibid, 180, 845 (1949)

Problem Code: X.R.4 (Hematology)

Section Code: 3351

Author: Marylou Ingram

Current Status of Studies of the Occurrence of Unusual Lymphocytes in the Blood of Cyclotron Personnel

The occurence in the peripheral blood of lymphocytes with bilobed nuclei has been noted in personnel employed in various capacities at the 130" Cyclotron Laboratory in Rochester, New York. Prior to completion of the cyclotron, a total of three such cells was observed during examination of approximately 170,000 leukocytes from 44 healthy individuals, and not more than one lymphocyte with a bilobed nucleus was found in the combined smears from any member of the group. On this basis the normal incidence in man has tentatively been estimated at approximately 1/50,000 leukocytes.

Three months after the cyclotron was put into operation the incidence of the cells in the same group was approximately 10/50,000 leukocytes, representing

a ten-fold increase based on the examination of approximately 200,000 cells. In two individuals accidentally exposed as a result of starting out of the back door of the shop towards the cyclotron while the cyclotron was running, the incidence of lymphocytes with bilobed nuclei was 20/50,000 leukocytes. In the one man who momentarily reached a point just beyond the end of the protective earth and concrete dike between the shop and the cyclotron, the incidence was 28/50,000 leukocytes, and on the day after the incident he had four lymphocytes with bilobed nuclei in 1,944 leukocytes, an incidence corresponding to 103/50,000.

In order to check the relationship between the increased incidence of abnormal lymphocytes and exposure to radiation from the cyclotron, three dogs have been studied before and after brief exposure. Each dog had complete daily blood counts for approximately two months prior to exposure, and during this time dogs were chained by a six foot chain in their assigned positions outside the cyclotron building several times when the cyclotron was not running in order to rule out the possibility that extraneous factors might be responsible for any observed increase in the incidence of the abnormal lymphocytes. Two of the positions chosen corresponded to the positions occupied by the two men who had been exposed accidentally. The third position was nearer the cyclotron building. Three nuclear track plates were attached to the collar of each dog. On August 22nd the dogs were chained in their respective positions for 30 minutes while the cyclotron was running, representing an increase of 10x - 15x over the exposure period of the two men. Although analysis of results is not yet completed it is possible to demonstrate a definite increase in the incidence of lymphocytes with bilobed nuclei. Whereas only one such cell was found among the 371,994 leukocytes from the combined smears of the three dogs during the control period, 22 were found

among the 420,000 leukocytes during the first week after exposure. It appears that the cells first occur in the peripheral blood one to two days after exposure, and are noted very infrequently after the first post-exposure week.

The exposed dogs were followed for approximately one month after exposure and appeared to have returned to normal as regards the lymphocytes. The animals were then exposed a second time. Results from examination of post-exposure smears from the second exposure are currently being interpreted. It is expected that a third exposure will be carried out using the same animals after they have recovered from the second exposure.

The increased occurrence of lymphocytes with bilobed nuclei appears to be an unusually sensitive indicator of exposure to radiation originating from the cyclotron. In the case of the accidental exposure of the two men it is definitely felt that routine monitoring devices such as film badges would almost certainly have failed to indicate any exposure. Similarly, routine hematological examinations are relatively insensitive to exposures of similar magnitude.

Problem Code: X.R.6

Section Code: 3380

Author: James G. Wilson

Effects of X-Irradiation on Embryonic Development in the Rat

In previous progress reports and in a manuscript now in preparation, a method was described whereby certain of the embryos in pregnant rats could be subjected to direct irradiation while the remaining embryos, together with the mother, were shielded from irradiation. Pregnancy was interrupted at various post-irradiation intervals (24, 48, 72, 96 or 120 hours) by killing the mothers; and

the uterus was examined for living, dead and resorbed embryos. Surviving embryos were weighed, measured and prepared for histologic study.

The first phase of the problem dealt with the effects of irradiation on the 10th day of gestation with dosages of 50, 100, 200 or 400 roentgen units administered at a single exposure. Three to six embryos in each of 37 pregnancies were exposed. Thus, of 335 embryos, 138 were irradiated and 197 were shielded to serve as controls. The effects of this type of irradiation fell into three general categories: (1) death of the embryo during the post-irradiation interval, (2) decrease in the rate of overall body-growth, and (3) abnormal growth, that is, specific retardations and malformations.

Dosage with 50 r on the 10th day was found to be ineffective, since embryos exposed to this dosage did not differ from their littermate controls in any of the above mentioned respects when pregnancy was terminated one to five days later. This was substantiated in three litters allowed to go to term; the previously exposed newborns were in no way different from their unexposed siblings. Furthermore, no evidence of impairment in the germ plasm was observed when the irradiated offspring were bred by brother-sister matings through three generations.

Exposure to 100 r on the 10th day, however, altered development in several respects. The rate of intrauterine death was increased from 14.2% in non-irradiated embryos to 25.5% in irradiated embryos. In the exposed animals which survived the rate of overall growth during the 5-day post-irradiation period was reduced by an average of 13%. The course of development was also appreciably altered. Specific retardation was noted in the central nervous system, the genito-urinary tract, and the heart of about one-third of all embryos receiving this dosage. Frank malformations were prevalent only in the eye; but some form of ocular anomaly was found in 68% of irradiated embryos. Malformations in other organs

were rare and limited to the brain and urinary tract. Two litters, some embryos in which received 100 r, were allowed to go to term. Birth weight was lower and postnatal death rate was higher in the exposed young than in their non-exposed littermates. Approximately one-half of the survivors were found to be permanently blind due to anophthalmia or extreme microphthalmia. Irradiated animals, including the blind ones, were bred by brother-sister matings through three generations; but no hereditary defects were detected.

Irradiation with 200 r on the 10th day was considerably more damaging than treatment with 100 r. Intrauterine death by or before the 5th post-irradiation day occurred in 36.5% of the embryos receiving this dosage, as compared with 14.2% of control animals. During the same period the rate of overall growth in the exposed animals that survived was approximately 35% below that of unexposed animals. Alterations in the course of development followed the same general pattern as was noted after 100 r, but both specific retardations and malformations were more extreme in degree and more prevalent in occurrence after 200 r. In addition to a high incidence of severe ocular anomalies, malformations were observed in the brain, heart, kidneys, aortic arches, and appendages in several instances. Also striking was the effect of this dosage on hematopoiesis in the liver. In many of the irradiated embryos the liver was almost devoid of blood-forming elements, while in control animals of this age the liver was the site of great hematopoietic activity.

A dosage of 400 r on the 10th day proved to be invariably fatal to the exposed embryos within the first 24 hours after irradiation.

The results summarized above do not comprise an exhaustive study of the effects of x-irradiation on the 10th day, but they are sufficient to establish a general pattern of response to various dosages of x-rays at this stage of develop-

ment. With the methods thus developed and using the results already obtained as a standard of comparison, it is now proposed to investigate the effects of x-irradiation at other stages of development. Accordingly, several embryos in each of 7 pregnant rats have been subjected to x-rays on the 9th day of gestation. As yet the number of animals studied is too small to permit definite statement of results. Disregarding the pattern of the response, however, it is already apparent that any dosage administered on the 9th day is more damaging than the same dosage given on the 10th day.

PROGRAM I.R.

BIOLOGICAL EFFECTS OF EXTERNAL RADIATION (INFRA-RED AND ULTRA-VIOLET)

Problem Code: I.R.1 (Flash Burns)

Section Code: 2602

Authors: H. D. Kingsley, Lewis Hogg, H. E. Pearse, V. DeLalla, R. M. Blakney, T. D. Davis

The continuation of the studies of flash burns has brought forth a number of problems which require solution in order to fully understand the relationship of physical agent producing the burn and the resultant lesion. Experimentation has been proceeding in two directions; first to improve the source of high intensity energy and determine its various characteristics, and second, to study the effects of the measured amount of incident energy on an experimental animal.

In previous reports the problem of providing a high intensity-short duration source of heat was discussed. The use of a high intensity carbon arc as the source of energy was established as the most adaptable to laboratory use. Refinements of this apparatus were necessary to obtain accurate measurements and to improve efficiency. The enlargement of the source is equally important in order to produce as large an area burn as possible. This aim is sought to more nearly duplicate in the laboratory the type of injury produced by non-ionizing radiation from an atomic bomb blast.

In evaluation of effects of flash burns, it has become important to study the experimental animals more completely. Standardized values of various physiological measurements are essential in order to evaluate effects of burns. Since it has been established that infection plays an important role in healing of burns, bacteriological studies have been undertaken. Histo-pathological

studies have been continued.

Methods

A. Physical aspects

1. Source of energy.

Two sizes of searchlights are in use; a 24" U. S. Navy, Model 1942 Searchlight, and a 60" U. S. Army, Model 1942 Searchlight. The parallel lig emerging from the open drum face of the searchlight is collected by a parabo reflector, a duplicate of the mirror within the searchlight drum, and brough a focus. Reflectors are front-surfaced with rhodium plate.

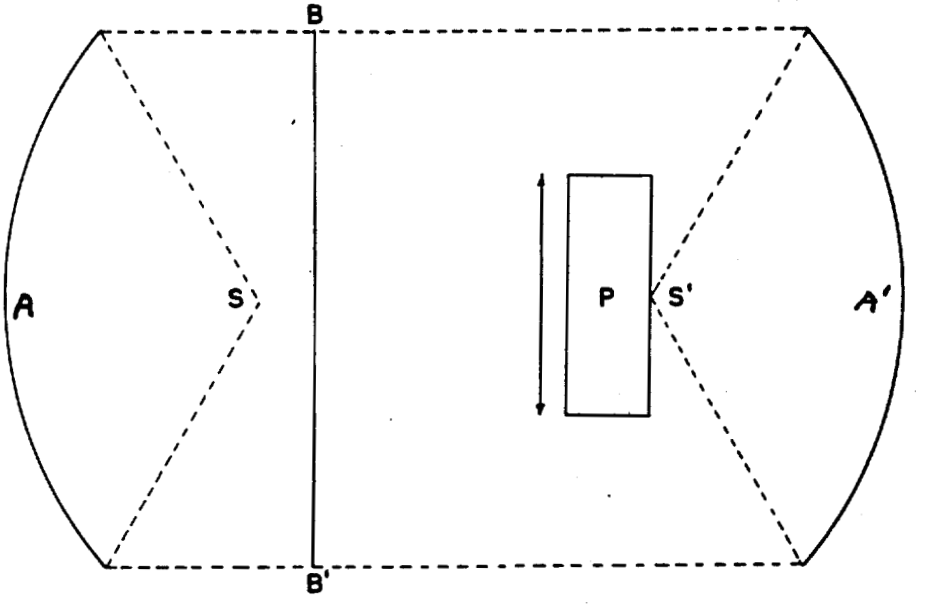

FIG 1 - SCHEME FOR OPTICAL SYSTEM OF 60 INCH SEARCHLIGHT

In Figure 1 a schematic diagram is shown. S and A are the carbon arc reflector, respectively, of the searchlight. The near parallel beam of ligh intercepted by a second parabolic reflector, A', which refocuses the beam at The subject is passed between the two mirrors at 90° to the light rays in su manner that the area to be burned passes through S'. Means are provided to reflector A' in any direction for obtaining accurate focus.

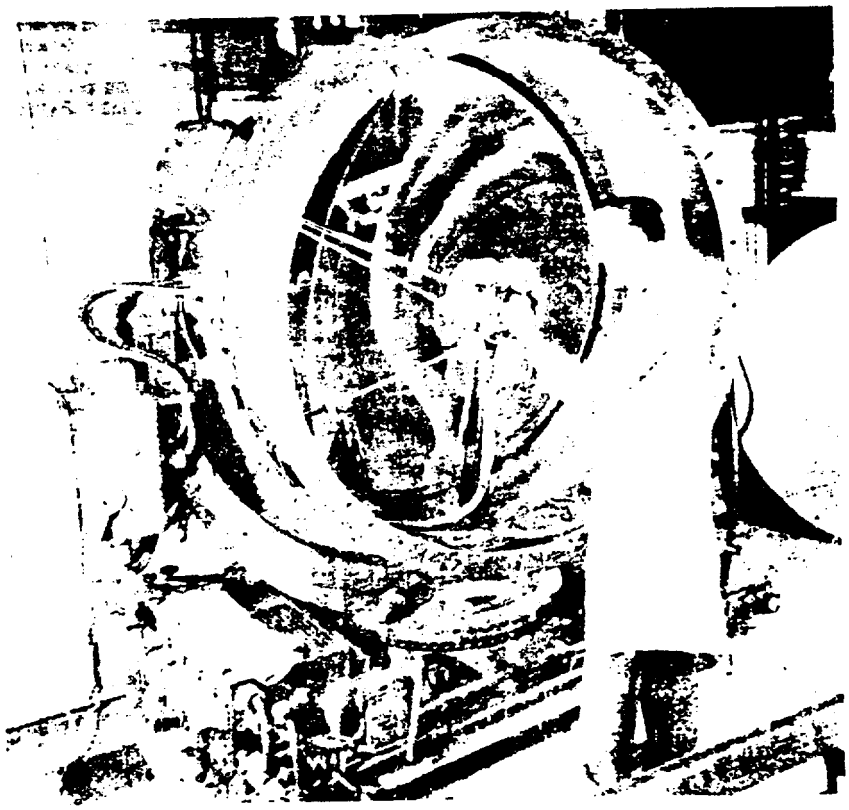

Figure 2

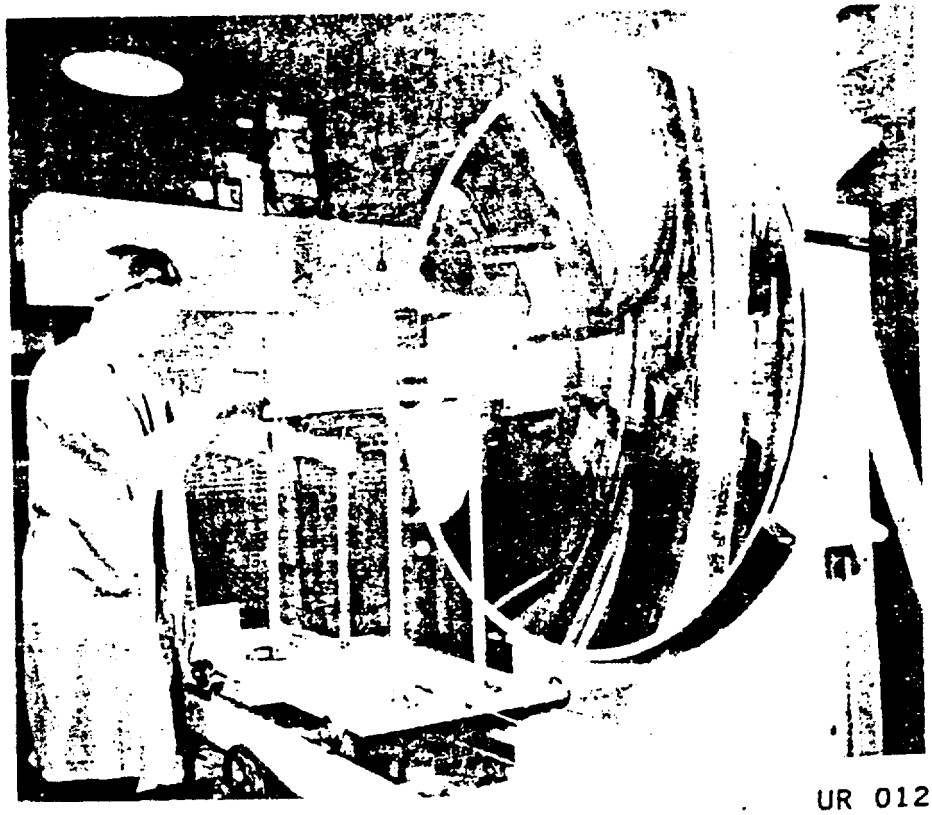

Figure 3

The beam is focused on the subject by placing a mask over the drum face (B') through which is punched four holes, 90° apart and equidistant from the center. Images from these four holes are superimposed directly on the subject. When the mask is removed, the subject can be passed through the point S'. The searchlight and reflector are shown in Figures 2 and 3 (Page 37).

A rectangular aperture in a sheet of transite on the side of the box facing the second reflector provides the opening for exposing the flank of the animal to the focused beam of light.

2. Energy measurements.

a. The Copper Sphere Calorimeter.

The Copper Sphere Calorimeter is an energy integrating device. It is designed to measure the total integrated energy of a pulse of radiant energy of high intensity and short duration. It is a non-selective receiver.

Figure 4 (Page 39) gives a schematic diagram of the instrument. C is a thin-walled copper sphere, which is made by spinning two hemispheres and soldering them together. There is a $\frac{1}{2}$" opening in the sphere for the introduction of energy. The inside of the sphere is blacked with zinc black. The method of blackening is described by W. E. Forsythe (1). The only modification of this method is that the filament must be made with long enough leads to get the filament through the opening of the sphere and position it in the center of the sphere.

Four constantin leads of 3 mil diameter are soldered to the outside surface, 90° apart and 90° from the opening. A 3 mil copper lead is soldered at a position 180° from the opening. A minimum amount of solder is used, both for soldering the leads and for soldering the two copper hemispheres.

Figure 4 (Page 39)also shows the measuring circuit, which has been modified in later measurements as in Figure 5 (Page 39). Here, the emf generated by the

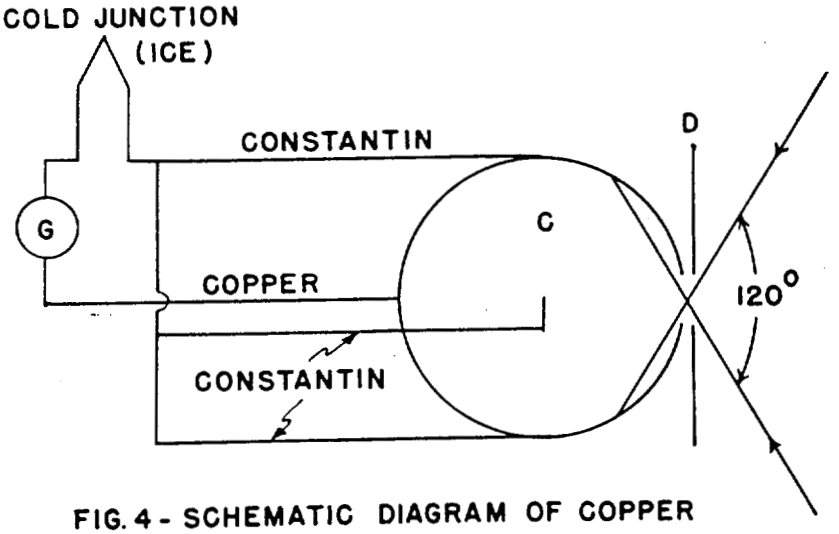

FIG. 4 - SCHEMATIC DIAGRAM OF COPPER SPHERE CALORIMETER.

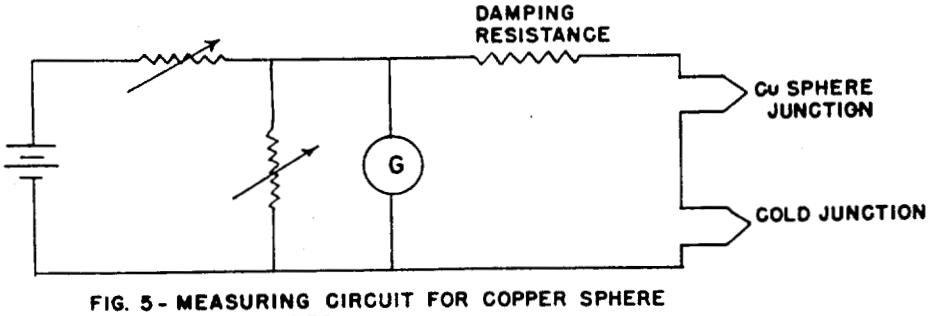

FIG. 5 - MEASURING CIRCUIT FOR COPPER SPHERE CALORIMETER.

initial temperature difference between the junctions on the sphere and the cold or reference junction, is bucked out with an opposing emf. The purpose of this is to make it possible to adjust the galvanometer without twisting the suspension excessively. This makes it possible to take more readings in any period of time because one does not have to wait for the sphere to cool down after each reading.

The sphere is mounted between six lucite points, which are so made to hold the sphere firmly by spring tension. The sphere and mounting are contained in a lucite box which is painted with aluminum paint to make it opaque.

The front side of the box has a $2\frac{1}{2}$" diameter hole, outside of which is a $\frac{1}{4}$" brass plate which also has a $2\frac{1}{2}$" diameter hole which coincides with the hole in the lucite. The hole in the brass plate is threaded to accomodate diaphrams of various sizes. Figure 6 (Page 41) shows the mounted copper sphere before it is inserted in the box. Figure 7 (Page 41) shows the instrument assembled.

Figure 8 (Page 42) shows a cross-section of the instrument, showing the construction of the diaphram. This is a brass disc with a hole in the center. The front of the disc is hollowed out in the shape of a 130° cone, in order to accommodate the large angle of the refocused searchlight beam. The rear surface of the disc is turned to a cone having an angle of 170°. This much is then chrome-plated. A plane lucite disc, silvered (by evaporation) on both sides, is attached to the rear surface of the disc as shown in Figure 8 (Page 42). This is to prevent the secondary radiation of the diaphram from striking the sphere.

The Copper Sphere Calorimeter is calibrated by sealing the opening and immersing the sphere in an agitated water bath. A standard thermometer, graduated in 0.1° C, measures the temperature of the water bath. The temperature of the bath is varied by adding small amounts of hot water. Due to the low specific heat of copper, the sphere assumes the temperature of the water almost immediately.

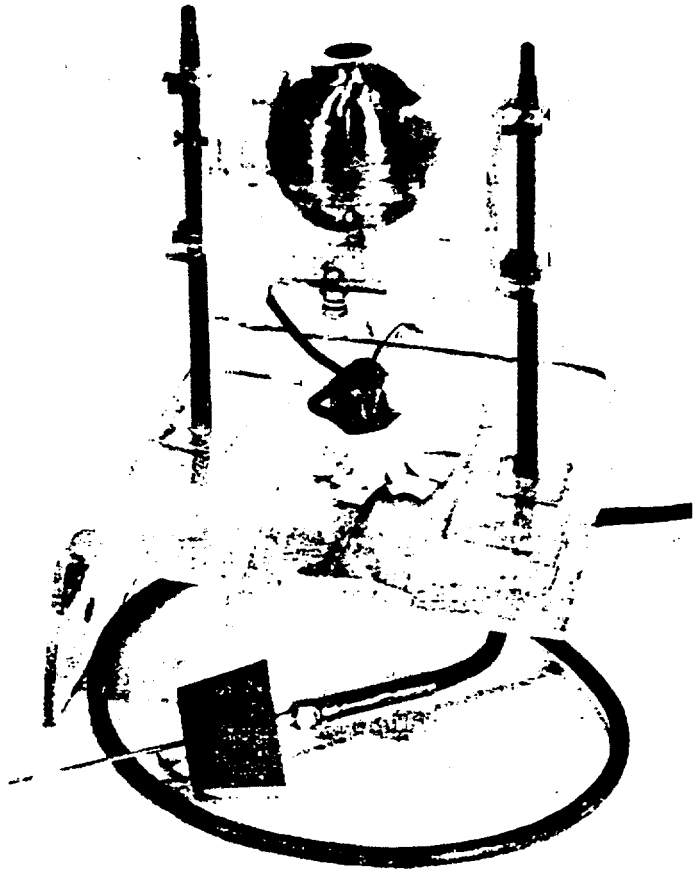

Figure 6

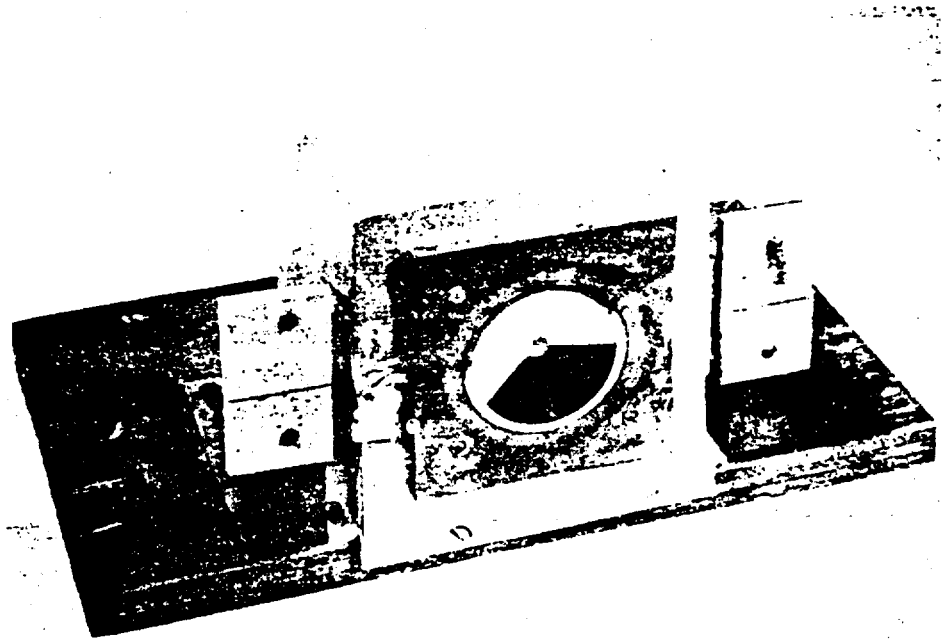

Figure 7

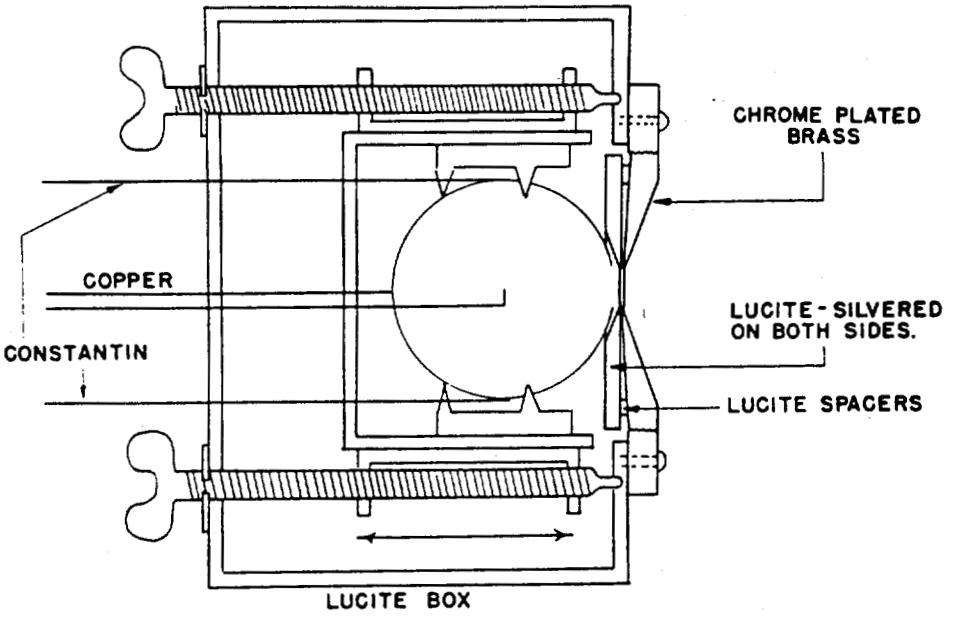

FIG. 8 - CROSS SECTIONAL VIEW OF COPPER SPHERE CALORIMETER

The temperature vs. galvanometer deflection is plotted. This is found to be a linear function over a wide range.

Knowing the weight of the sphere and its specific heat, a calibration curve can be plotted in terms of the energy absorbed and the emf generated.

b. Platinum-Platinum Rhodium Thermocouple probe.

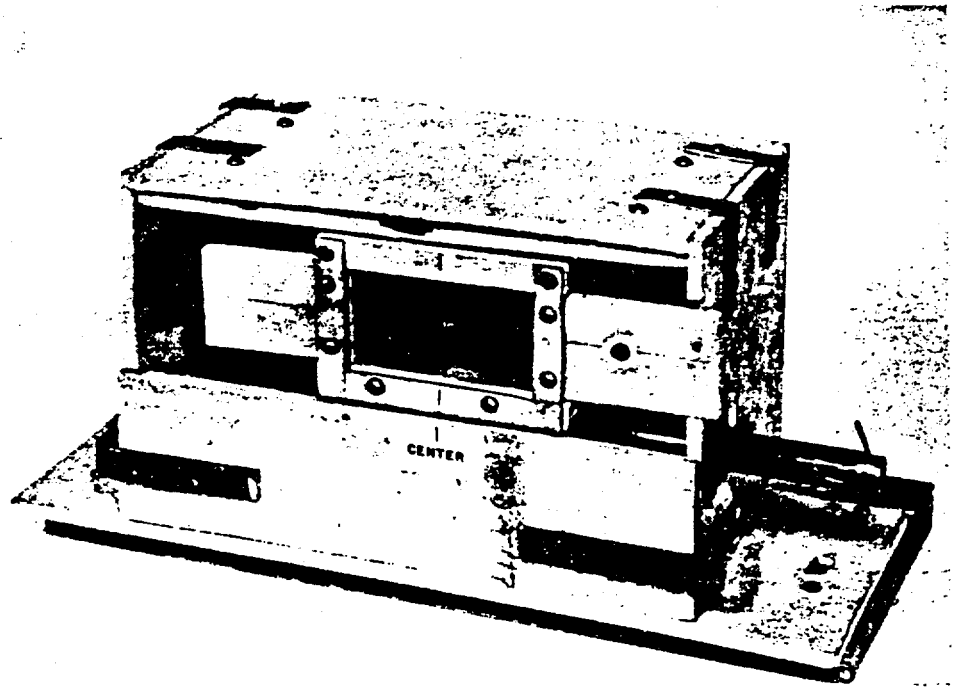

Figure 9

Figure 9 above shows this probe. A platinum-platinum rhodium thermocoupl is mounted in such a fashion that it can be moved in very small increments, the total travel being 5.6 cm. The reference junction is placed in the shadow, and its temperature is assumed to remain reasonably constant.

The output of the thermocouple is fed directly to a Brown Recording Potentiometer. The thermocouple was moved 0.035 cm at a time and allowed to stand for 30 seconds before moving again. The average of each 30 second reading

was taken, and the data plotted as percent total intensity vs. distance from the center of the spot.

3. Experimental animals.

Young Chester White pigs have been used. Ages vary from 2-6 months. Weights vary from 15-40 pounds. Both sexes are represented.

After procuring animals a period of at least two weeks is used to make observations as to weight change, hematology, blood chemistry and bacteriological flora on the caged animal.

Pigs are fed a ration of Purina "Sow and Pig Complete Ration" which contains 1500 cal. per pound and a digestible 1200 cal. per pound. This diet contains adequate amounts of essential amino acids, fats, carbohydrates, minerals and vitamins.

In preparation for exposure to irradiation, the animals are anesthetized with veterinary nembutal, .065 gms per 5 pounds given intravenously. Supplemental nembutal is frequently necessary. The hair is carefully removed by clipping closely with electric clippers. The pig is placed prone in the transite box and adjusted to present the flank as a plane surface behind the aperture in the transite.

The speed of the cart traversing the beam can be varied to change duration of exposure of 1 sq. cm. from .26 secs. to .04 secs. with the present equipment.

After exposure, drawings and photographs are made of the resultant burn. A biopsy of the burn and adjacent tissue is made within the first hour. Frequent observations of appearance of burn and its pathology are made. Hematological and blood chemical determinations are carried out in subsequent periods.

4. Pathology.

Biopsies of the burns and adjacent normal skin are taken immediately after

the exposure and at intervals of 24 hours thereafter. Strips 4-5 mm in width, 1 cm. deep and 4-6 cms in length are removed, attached to a glass slide and fixed in Bouin's solution. Fixation to a glass slide by a suture was found to insure uniform sections for study. Standard paraffin imbedding and microtome sectioning technique is used to provide sections for hematoxylin and eosin staining.

5. Hematological studies.

Determinations of WBC, RBC, sed. rate, hematocrit and Hbg have been made. Red and white blood counts are done by standard hemacytometers in duplicate. Hemoglobin is determined in the Sahli apparatus and checked by Klett-Summerson photo-electric colorimetric method. Sedimentation rates and hematocrit determinations are done in Wintrobe tubes by standard clinical methods.

6. Blood chemistry.

Determination of total protein, albumin, globulin, urea nitrogen, chloride, icterus index of serum and blood hemoglobin have been made.

Total protein is determined by the micro-Kjeldahl method as modified and described by Howland (2). An error of less than 1% has been obtained in determinations of standard samples of ammonium sulfate by this method.

A/G ratio of serum is determined by methanol fractionation as described by Pillemer and Hutchinson (3). The albumen fraction is measured by Kjeldahl digestion and the globulin value given by subtracting amount of albumen from the value for total protein.

Values for chloride concentration in serum are obtained by the mercuric nitrate method as described by Schales and Schales (4).

Blood urea nitrogen is measured by the standard urease method of urea digestion.

Icterus index is determined by comparing a potassium chromate color

standard with diluted serum in the Klett-Summerson photo-electric colorimeter.

Hemoglobin concentration in whole blood is determined by comparing the optical density of a standard acid hematin solution and acidified, diluted whole blood in the Klett-Summerson photo-electric colorimeter.

7. Bacteriology.

Standard bacteriological methods have been used to determine the presence and analyze the types of organisms present as contaminants of skin and mucous membranes of pigs before production of burns. Subsequent to burn injury, cultures are taken of the burn and biopsy wounds.

Results

A. Physical characteristics of source.

1. Losses involved in the present set-up.

The two reflectors, A and A', are rhodium plated, and are at most only 65% efficient. Since they are not perfectly parabolic, there is also a small loss due to the fact that the beam projected from the primary reflector is not quite parallel, about 6% not being collected by the second reflector.

There is a further loss due to the fact that about 30% of the energy delivered to the arc is in the tail flame which cannot be collimated. In the case of the 24" searchlight, the necessary size of the transite box for holding the subject presents an opaque surface to the beam projected from the primary reflector whose area is 50% of the beam area. For the 60" searchlight, this loss is reduced to 9%.

For the 24" searchlight, the energy delivered to the subject is at most 25% of the energy emitted from the positive crater. In the case of the 60" light, the energy delivered to the subject is 45% of the energy collected from the positive crater.

2. The following table gives the results of the measurements on the 24" Carbon Arc set-up taken with the copper sphere calorimeter with the various diaphrams.

TABLE I

Diaphram area cm^2	Exposure time sec	Energy Measured cal.	Flux thru Diaphram cal/mm^2 sec	Flux thru Diaphram $watts/mm^2$
0.974	0.16	7.1	0.46	1.9
0.974	0.26	13.0	0.51	2.1
0.546	0.16	4.6	0.53	2.2
0.546	0.26	8.4	0.59	2.5
0.317	0.16	2.3	0.45	1.9
0.317	0.26	4.5	0.55	2.3

3. The following table gives the results of the measurements on the 60" Carbon Arc set-up taken with the copper sphere calorimeter with the various diaphrams.

TABLE 2

Diaphram area cm^2	Exposure time sec	Energy Measured cal.	Flux thru Diaphram cal/mm^2 sec	Flux thru Diaphram $watts/mm^2$
0.974	0.19	15.6	0.84	3.5
0.974	0.32	26.5	0.85	3.5
0.546	0.19	9.7	0.94	3.9
0.546	0.32	16.5	0.95	3.9
0.317	0.19	5.1	0.85	3.5
0.317	0.32	8.9	0.88	3.7
0.062	0.19	1.3	1.10	4.6
0.062	0.32	2.1	1.06	4.4

2. The following table gives the results of the measurements on the 24" Carbon Arc set-up taken with the copper sphere calorimeter with the various diaphrams.

TABLE 1

Diaphram area cm^2	Exposure time sec	Energy Measured cal.	Flux thru Diaphram cal/mm sec	Flux thru Diaphram watts/mm^2
0.974	0.18	7.1	0.41	1.7
0.974	0.29	13.0	0.39	1.6
0.546	0.13	4.6	0.65	2.7
0.546	0.21	8.4	0.73	3.0
0.317	0.10	2.3	0.66	2.8
0.317	0.17	4.5	0.84	3.5

3. The following table gives the results of the measurements on the 60" Carbon Arc set-up taken with the copper sphere calorimeter with the various diaphrams.

TABLE 2

Diaphram area cm^2	Exposure time sec	Energy Measured cal.	Flux thru Diaphram cal/mm^2 sec	Flux thru Diaphram watts/mm^2
0.974	0.18	15.6	0.91	3.8
0.974	0.29	26.5	0.94	3.9
0.546	0.13	9.7	1.4	5.8
0.546	0.21	16.5	1.4	5.8
0.317	0.10	5.1	1.6	6.7
0.317	0.17	8.9	1.7	7.1
0.062	0.05	1.3	4.7	19.6
0.062	0.07	2.1	4.6	19.3

4. The curve of Figure 10 below shows the relative intensity of the energy as a function of the distance from the center of the spot. These measurements were taken directly in the focal spot.

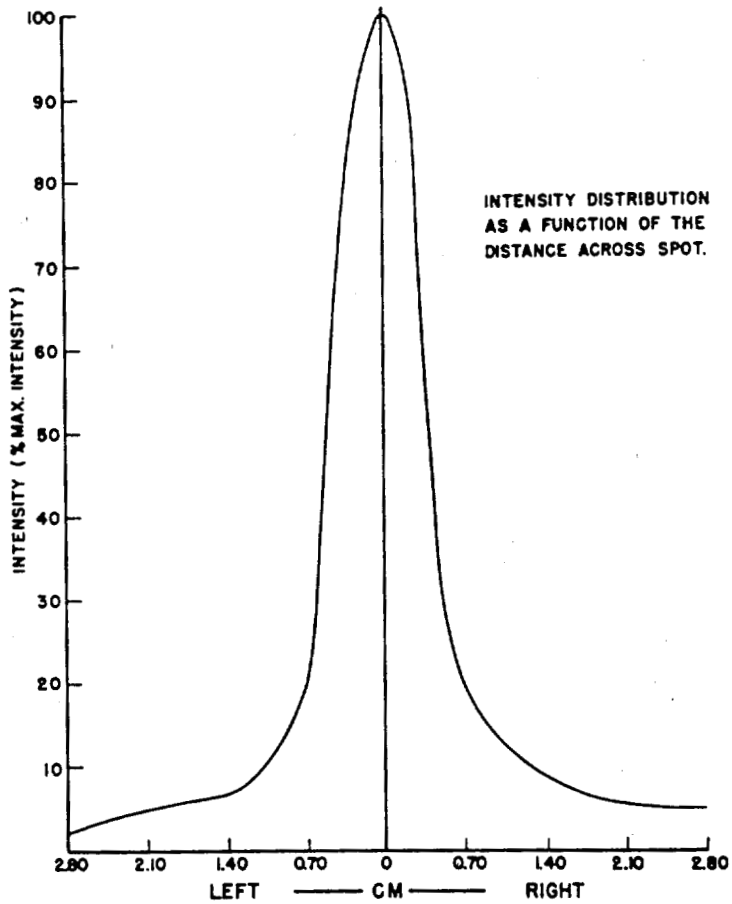

Figure 10

B. Physiological Results

1. Burn production.

The lesions observed consisted of 3 discrete zones, 1. a central zone colored gray white with a roughened surface showing irregular carbonization of the skin and hair and occasional delicate blebs with dry bases, 2. a surrounding distinct zone of erythema 2-5 mm wide and 3. a transitory stellate flare reaction

20-30 mm wide. The central zone is approximately 1 x 10 inches in size which corresponds to the measurement of the slot in the transite. An infrequent finding not seen previously was the appearance of a discrete elevated edematous area beneath the erythema and flare zones that surrounded the lesion. This subsided one hour after exposure.

At 24 hours, the central zone was a pale violaceous gray in color, elevated, puckered and soft. At this time the blebs were nearly completely denuded, and dry fissures were present. The erythematous border was narrowed (1-3 mm) and red-purple in color.

There was no further noticeable change until 72 hours, at which time the central zone appeared more as an eschar with brown pigmentation beginning. The immediately adjacent area also showed a brown pigmented color rather than erythema.

Eschar formation was progressing at 5 days and was fully developed at 7-9 days. The eschar was leathery, brown and black and underwent partial peripheral detachment. Regenerated pale and translucent epithelium was present beneath the detached periphery. Progressive detachment and shrinkage occurred and was completed in approximately 14 days. The eschar was removed by activity of the animal in his cage. The underlying epithelium was pink, thin and translucent. Hair regeneration was discernible in spotty areas.

At the present time, sections have not been returned for microscopic study. For this reason the microscopic observation will be included in a subsequent report.

2. Hematology.

Hematological determinations were performed on six pigs of the Chester White strain. The animals were studied for a 6 week period, and approximately

30-40 determinations performed on each animal.

Throughout the studies the animals gained weight, remained free from infection, and appeared normal. The determinations were performed by the same technician using standard procedures. At the end of the investigative period, the maximal and minimal values were collected and recorded for all the animals. A mean was established for all the means of the various animals and this data is listed in tabulated form below:

	Maximal	Minimal	Range of Mean (6 animals)	Average Mean
RBC (Millions)	8.80	4.90	6.92-7.46	7.13
WBC	32,000	15,900	18,990-22,720	20,586
Sed. Rate (mm/hr.)	27.0	0	.97-3.92	1.72
Hematocrit (%)	51.0	37.0	39.5-44.3	42.5
Hbg (Gms)	13.70	10.12	11.10-12.46	11.66

The study of hematological effects of burns in these animals is in progress.

3. Biochemistry.

Values for total protein, albumin, globulin, blood urea nitrogen, chloride, icteric index and hemoglobin in the serum were collected. The determinations were done on Chester White pigs, the same animals used for the hematological determinations. The maximal and minimal values and means for the group were established. They are tabulated as follows:

BLOOD CHEMISTRY VALUES

	Maximal		Minimal	Mean (6 animals)	Average
Total Protein gms %	8.28	-	5.26	6.06-6.93	6.31
Serum Albumen gms %	3.05	-	1.62	2.68-2.80	2.72
Serum Globulin gms %	4.90	-	2.87	3.16-3.99	3.55
Serum Chloride m eq.	149.7	-	101.2	112.9-125.5	117.7
Blood Urea Nitrogen mg %	16.40	-	1.60	10.0-14.9	11.24
Icterus Index	16.06	-	1.46	3.81-5.20	4.66
Serum Hemoglobin gms %	0.56	-	0.03	.16- .32	.23

Insufficient data have been obtained for evaluating the effects of burns on the blood chemistry of the animals. These effects are being studied.

4. Bacteriology.

Studies have been pilot in nature. The apparent normal flora of the pig is not significantly different from his surroundings.

Table 3 (Page 52) shows the various organisms found in samples from the pig.

Discussion and Conclusions

A. Physical characteristics of energy source.

The flux for two different times of exposure through the same diaphram area agree fairly well as the last two columns of Tables 1 and 2 (Page 47) show.

The agreement is better in Table 2 which is the data from the 60" searchlight arc. The most probable reason for this is as follows.

TABLE 3

Pig No.	Where Taken	No. Colonies Obtained	Colony Types Obtained	Identification and No. of Various Colonies to Date
25	Dry swab off side	8	Micrococcus Short chain Streptococcus Sarcina	1 strain Sarc lutea 2 strains other type Sarcina 2 strains Micrococcus luteus 1 strain other type Micrococcus 1 Gram ⁄ rod 1 short chain Streptococcus
25	Dry swab lip folds	None	_____	_____
27	Blood (not sterilely drawn)	2	Micrococcus	2 strains
28	Dry swab inner lip folds	None	_____	_____
28	Dry swab of bleeding site	None	_____	_____
29	Blood from infection site	6	Micrococcus Sarcina Short chain Streptococcus	2 strains Sarcina lutea 2 short chain Streptococci 2 strains Micrococcus
27	Dry swab from side	6	Sarcina Gram ⁄ rods Short chain Streptococcus	2 short chain Streptococci 2 strains Gram ⁄ rods (1 spore former) 1 strain Sarc lutea 1 strain other type Sarcina
28	Dry swab lip folds	6	Sarcina Micrococcus	1 probable short chain Streptococcus 2 strains Sarc lutea 1 strain Micrococcus candidus 2 strains other type Sarcina

When one observes the two arcs in operation, it is readily noticeable that the 60" arc is the steadier of the two. The 24" arc sputters considerably, and the current through the arc varies as much as 3%. This is a non-periodic variation. The feeding mechanism for the 24" arc is not dependable for maintaining a constant arc length for any period of time. This requires periodic manual adjustment. The light output from the arc is very sensitive to the position of the positive crater with respect to the focal point of the reflector. Very slight changes in position of the positive crater produce marked changes in the intensity at the second focal point. This was noted in exploring the focal spot with the platinum-platinum rhodium thermocouple.

There is a periodic variation of the flux through the second focal point of about 3-4%, the period of which corresponds to the time of one revolution of the positive carbon. There are also random variations as great as 10%, the reason for which is not clear. Then there are the variations due to the necessity for manual adjustment of the position of the positive crater, but these are kept to a minimum and probably do not amount to more than 2%.

The feed rate of the 60" light is automatically controlled by a thermostat. Although the thermocouple probe has not been used to explore the second focal spot of this light as yet, it is expected that the variation in flux will be much less. The arc is much quieter and more steady than the smaller one. This is born out by the figures in the last two columns of Table 2 (Page 47), which show close agreement for the flux for two different exposure times through the same diaphram area.

The carbon used in the 24" light is an experimental high intensity 10 mm positive which was obtained from the National Carbon Co., and is rated at 145 amperes and 80 volts. The carbon used in the 60" light is the standard 16 mm

searchlight carbon which is rated at 150 amperes and 80 volts. The increase in flux from the 60" light is therefore due primarily to the reduced loss due to the subject in the parallel beam.

The standard positive carbon for the 24" light is the 10 mm searchlight positive, rated at 100 amperes and 90 volts. No accurate measurements have been taken with this carbon due to the fact that the Copper Sphere Calorimeter was not sufficiently developed at that time, and since the advent of the experimental high intensity positive, no use has been made of this carbon.

Table 4 below gives the energy delivered to the subject to be burned. The area considered is one square centimeter, so that when one square centimeter of the subject passes completely through the spot, the energies listed in Table 4 fall on that area in the time of the exposure. These values are taken from Tables 1 and 2 (Page 47) for the 0.974 cm^2 aperture, corrected to 1 cm^2. The value given for the standard 10 mm positive carbon used in the 24" light are the best available. They are included to show the improvement of the 10 mm experimental high intensity carbon over the standard carbon.

TABLE 4

	Energy delivered to 1 cm^2 of the subject in the focal spot cal/cm^2	
Time of exposure	0.29 sec	0.18 sec
24" carbon arc with standard 10 mm search light positive operated at 100 amps and 90 volts	8.7	4.9
24" carbon arc with 10 mm experimental high intensity positive operated at 145 amps and 80 volts	13.4	7.3
60" carbon arc with standard 16 mm searchlight carbon operated at 150 amps and 80 volts	26.5	15.6

Work on the Copper Sphere Calorimeter is still going on. Refinements are planned which should increase the accuracy. More detailed study of temperature distribution on the sphere and the losses involved are in progress.

[redacted]

At the present time no general systemic effects of the burns such as immediate or delayed shock have been noted. The relatively small surface area of the burn amy account for this. Plans have been made for redesigning the apparatus in order to provide facilities for relatively large surface area burns. A comparison can then be made between animals with areas of flash burns and similar areas of low temperature burns.

A mild to severe burn can be produced by intense light, generated from a carbon arc. Lesions produced are remarkably constant in gross appearance when the intensity of illumination and the duration of exposure remain constant. The variable appearance of the burn strip particularly in r egard to the amount of dry bleb formation and carbonization, is thought to be due to movement of the inadequately anesthetized pig. In this latter event, the animal in his attempt to withdraw from the source of injury, moves in and out of the focal spot of the light. Plans are made to more adequately anesthetize the animal and provide better fixation beneath the transite mask.

Since more accurate methods for measuring the incident energy are now available, assessment of biological effects produced by quantitative amounts of energy can be made. Measurements of threshold energy, i.e. that amount of energy necessary to produce trans epidermal necrosis, are contemplated.

Further studies of the normal bacteriological flora and its relationship to healing of burns are in progress.

Bibliography

1. Forsythe, W. E., Measurement of Radiant Energy, McGraw-Hill (1937)

2. Howland, J. W., unpublished data

3. Pillemer, L., and Hutchinson, H., J. Biol. Chem., 158, 299 (1945)

4. Schales, O., and Schales, S. S., J. Biol. Chem., 140, 879 (1941)

PROGRAM U.

URANIUM

Problem Code: U.4 (Fate)

Section Code: 3210

Author: C. W. LaBelle

The Distribution of Radioactivity in Pile Filter Dust as a Function of Particle Diameter

The so-called pipette-sedimentation technique for the measurement of particle-sized distributions has been available for some years (1-3) and has been used successfully (4, 5) to follow separation processes for the preparation of fractions of graded particle size. The method may be applied for the determination of the distribution of radioactivity as well as mass with respect to particle size, and hence of the specific activity of any given size component. The principle on which the method rests is the following.

Consider a cylinder of depth, P, containing a suspension of particles of diameter, d. Assume that a pipette is inserted to a depth, h, and a sample withdrawn. This sample may be presumed to come entirely from the element of volume $h = h + \Delta h$.

At any time, t, there will be the same number of particles in the element $h - \Delta h : h$ as in the element $h : h + \Delta h$, since the distribution of the particles is random. At some later time $t + \Delta t$, such that $v_d = \Delta h / \Delta t$, when v_d is the terminal velocity of the falling particles, all the particles in $h : h + \Delta h$ will have settled out of the element of volume, and will have been replaced by the equal number originally present in the element $h - \Delta h : h$. There is, therefore,

no change in the concentration of particles in the element of volume being sampled by the pipette. This relation obtains until a time t_d is reached, such that $t_d = h/v_d$; that is, until those particles originally at the upper boundary have had time to fall to the depth, h. At this time the concentration of particles in h : h + Δh falls to zero, where it remains for the remainder of the process.

The preceding assumes that the particles are monodisperse with respect to size. If the particles are polydisperse, with a maximum size, d_{max}, present, this maximum particle will have a maximum terminal velocity, v_{max}, and there will be a corresponding minimum time interval $t_{min} = h/d_{max}$ during which no change in the concentration of this or any smaller size will occur. This is of the greatest practical importance, since it indicates that a sample may be withdrawn from a suspension with a pipette without introducing errors due to settling provided only that the critical time, t_{min}, is not exceeded.

When a mixture of sizes are present in the suspension, there will be:

C_{v1} grams/cc of particles of diameter d_1 and velocity v_1

C_{v2} " " " d_2 " v_2

C_{v3} " " " d_3 " v_3

C_{vn} " " " d_n " v_n

AND

$$C_{v1} + C_{v2} + C_{v3} \cdots C_{vn} = \Sigma C_v = Co \qquad (1)$$

If Co is taken equal to 1, at time, t, the total concentration in the element h : h + Δh is given by:

$$C_t = 1 - \int_{v}^{v_{max}}{}_{h/t} C_v \qquad (2)$$

where $\int_{v = h/t}^{v = v_{max}} C_v$ is the total concentration of particles whose velocity is equal to or greater than the critical velocity h/t. The diameter of these

particles is, by Stoke's Law, equal to or greater than:

$$d = K\sqrt{h/t} \qquad (3)$$

Combining 2 and 3, we obtain:

$$1 - C_t = \int_{d = K\sqrt{h/t}}^{d = d_{max}} Cd \qquad (4)$$

This is the integrated form of the curve of distribution of mass with respect to particle size and may be plotted by the usual methods on probability paper. C_t is determined experimentally by evaporating successive samples and weighing the residue, so that the cumulative distribution curve for the mixture may be constructed. From this curve the differential curve may be derived, giving the fraction of the total mass of sample which is associated with each size of particle.

The method may be extended further by measuring the total activity present in successive samples instead of the total mass. In this case equation 4 becomes:

$$1 - A_t = \int_{d = K\,h/t}^{d = d_{max}} Cd$$

where A_t is the relative activity per cc at time, t. This function may also be plotted on probability paper, taking the probability scale as indicating the percentage of total activity rather than percentage of total mass, as before. The differential curve may again be constructed. This curve, and the corresponding curve for mass give the amount of activity associated with a given size of particle, and the amount of mass associated with the same size, from which the specific activity of that size particle may be calculated*.

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* For some purposes, it is not necessary to introduce Stoke's law into the calculation. Equation 2 may be employed throughout, so that size and activity are related to particles of stated terminal velocity without the necessity for any assumptions as to size or density of the particles.

Experimental. A glass cylinder was fitted with a rubber stopper containing a glass tube to guide a pipette to a reproducible point in the container. The container was filled with a suspension of radioactive dust in water, and samples were withdrawn at intervals. In one series a concentrated suspension (1%) was employed and total solids were determined in the samples, and in a second series a more dilute suspension (0.1%) was employed and total beta-activity was measured. Distribution curves were constructed for each series, and the results combined as described.

Results. Figure 1 (Page 61) shows the distribution of mass, and Figure 2 (Page 62) the distribution of radioactivity in the sample, while Figure 3 (Page 63) shows both curves plotted in the differential form. Table 1 (Page 64) tabulates the total mass, total activity, and specific activity associated with each velocity (size) of particle. Figure 4 (Page 65) shows the specific activity as a function of the terminal settling velocities of the particle. It will be seen that the radioactivity of the sample is largely associated with those particles whose settling velocity is 0.2 cm/min.

Discussion. The results described in a preceding Quarterly Report indicated that the dust sample from the pile filters is very complex biologically, so that the interpretation of an animal experiment is difficult. This complexity is probably related in part to the fact that the sample is, as shown here, non-homogeneous physically. It seems advisable, therefore, to attempt to isolate this fraction of high specific activity and to use this fraction rather than whole dust in any further biological experiments.

Summary. 1. A method is presented by which the distribution of mass and activity with respect to particle size may be determined.

2. Application of this method to the dust taken from pile filters indicates the presence of a fraction of relatively high specific activity which settles in

Figure 1. Distribution of Mass in Filter Dust

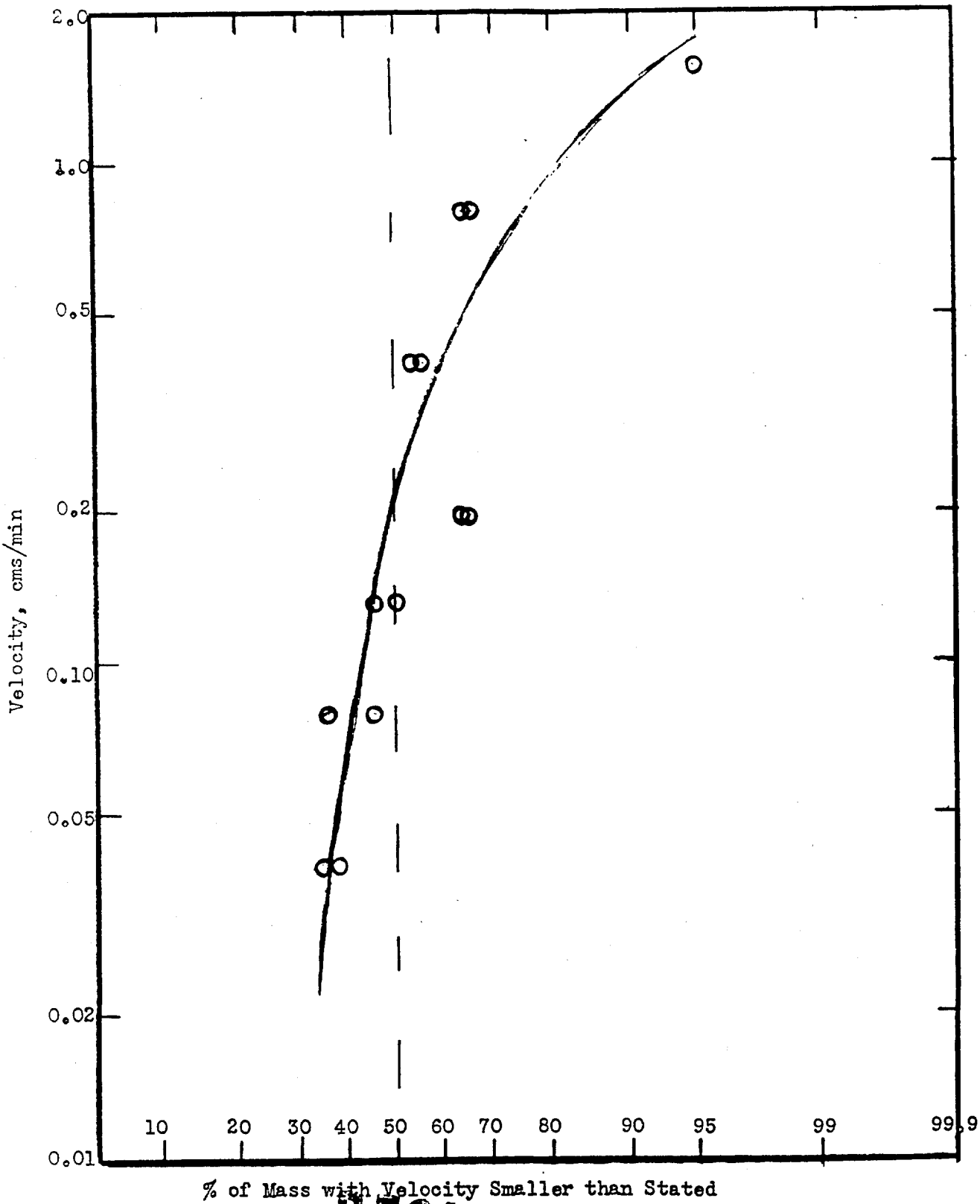
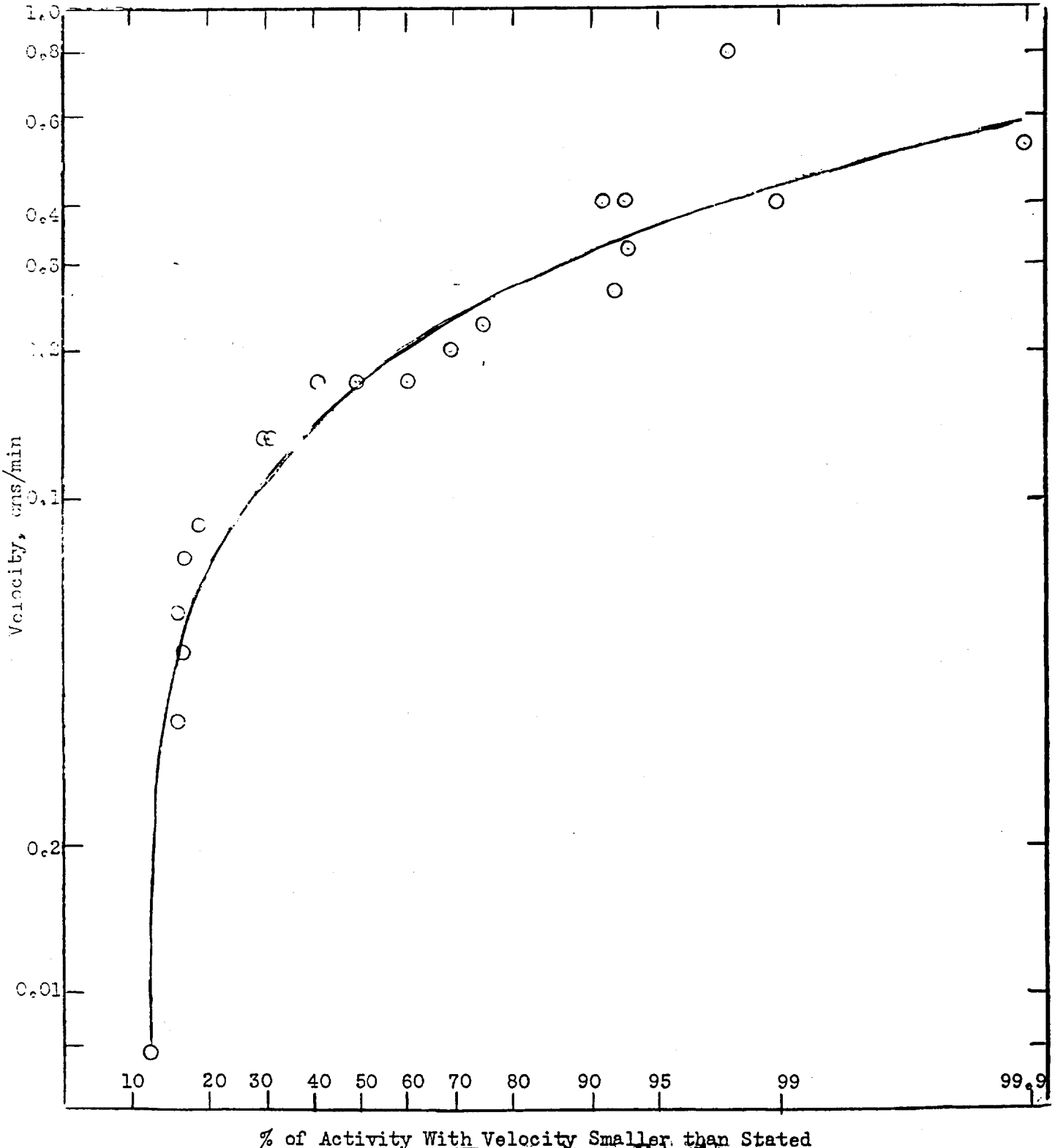


Figure 2. Distribution of Acitivity in Filter Dust



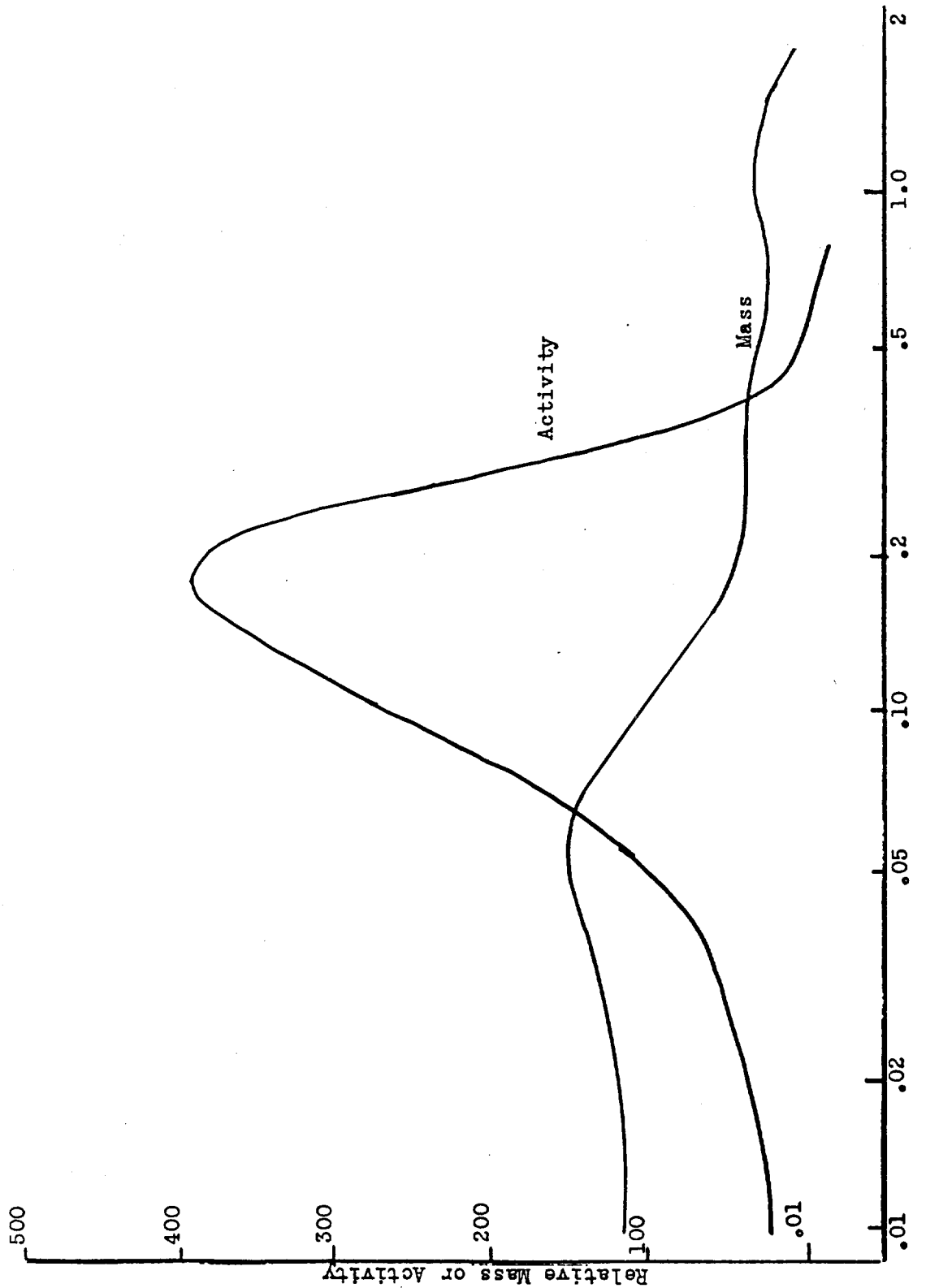


Figure 3. Distribution of Mass and Activity in Filter Dust

water with a velocity of 0.2 cm/min.

3. It is suggested that this fraction be isolated for further biological investigation.

TABLE 1

DISTRIBUTION OF MASS AND ACTIVITY WITH RESPECT TO SETTLING VELOCITIES

Terminal Velocity cm/min	$\Delta M/\Delta V$	$\Delta A/\Delta V$	Specific Activity
0.016	119	23	0.19
0.015	120	30	0.25
0.020	121	38	0.31
0.030	135	54	0.40
0.040	140	65	0.46
0.050	152	107	0.70
0.060	151	148	0.98
0.080	131	192	1.46
0.100	104	242	2.32
0.120	87	305	3.50
0.140	73	363	4.99
0.160	62	391	6.30
0.180	57	394	6.90
0.200	50	384	7.68
0.250	43	300	6.98
0.300	41	176	4.29
0.400	40	40	1.00
0.500	28	0	0.0

Bibliography

1. Robinson, G. W., J. Agr. Sci., 12, 306-21 (1922)

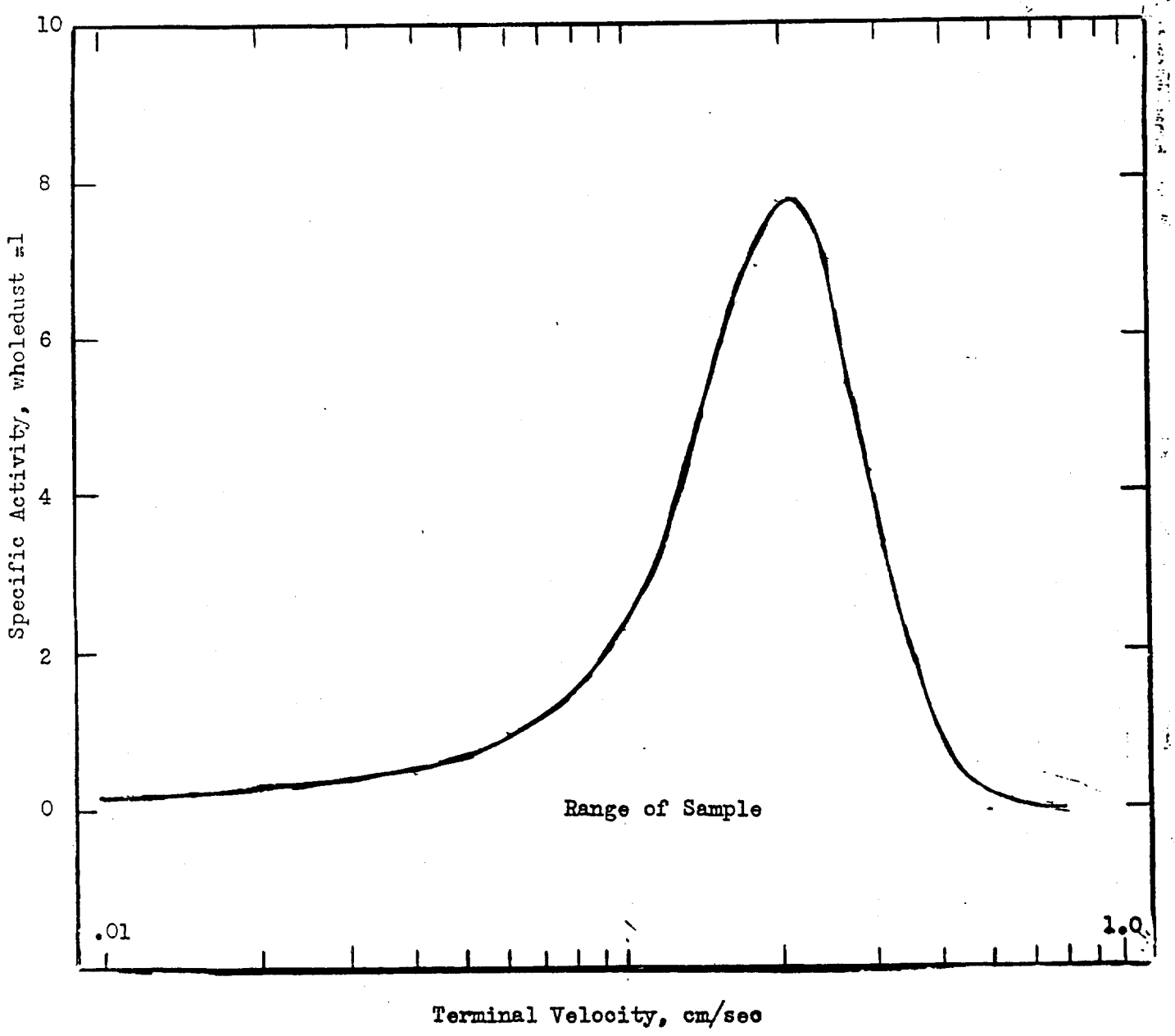
2. Andreasen, A. H. M. and Lundberg, J. J. V., Ber. Deut. Keram. Ger., 11, 249-62 (1930)

3. Berg, Soren, Kollardchem.-Beihiftc, 53, 149-376 (1941)

4. Burchfield, H. P., Gullstrom, K. D. and McNew, G. L., Ind. Eng. Chem., Anal. Ed., 20, 1168-72 (1948)

5. Gullstrom, D. K. and Burchfield, H. P., Ind. Eng. Chem., Anal. Ed., 20, 1174-77 (1948)

Figure 4. Specific Activity of Particles of Various Sizes



UNCLASSIFIED

PROGRAM Be.

BERYLLIUM

Problem Code: Be.2 (Toxic Effects)

Section Code: 3260

Author: R. E. Gosselin

Studies on the Phagocytosis of Inorganic Particles by Rabbit Cells in Vitro. Preliminary Notes

Introduction. Experiments now in progress have been designed to study the behavior of rabbit phagocytes when exposed in vitro to various insoluble dusts of industrial importance. Our principal interest is not the mechanism of phagocytosis but rather any changes in cellular physiology which may result from indigestible material which has been phagocytized. It is felt that significant changes may appear in the motility of the cell, the stickiness of its surface, its permeability to diffusible substances, its respiration, and its survival in vitro. Information of this character is not available in the literature, although such data should prove valuable in clarifying the pathogenesis of some of the pneumoconioses (especially silicosis and berylliosis) and in interpreting experimental results on the distribution of inorganic colloids injected in animals.

The following is a brief description of the experimental procedures which have been most satisfactory and of a few conclusions which must be regarded as tentative.

Materials. With a large needle, peritoneal fluid is aspirated from adult male rabbits 15 to 35 hours after an intraperitoneal injection of 300 ml of sterile 3% soluble starch solution in isotonic saline. The exudate contains large numbers of polymorphonuclear neutrophils (pseudo-eosinophilis) as well as a few

cells which are probably early (young) myeloid forms. A satisfactory exudate contains insignificant numbers of adult mononuclear cells and of red blood cells. The cells are removed from peritoneal fluid by slow centrifugation and resuspended in solutions of pure electrolytes. Over 95% of these exudate cells are alive, as judged by their failure to stain with dilute eosin (Schrek, Am. J. Cancer, 28, 386, 1936). These cells respire at rates comparable to those reported by Ponder and MacLeod (Am. J. Physiol., 123, 420, 1938). On a warm microscope stage, they are motile and readily phagocytize particles of quartz, beryllium oxide and uranium dioxide.

A second type of peritoneal exudate has been obtained from rabbits 4 days after the second of two intraperitoneal injections of 100 ml of 20% buffered gum acacia solution. These cells are exclusively mononuclear, a few small (lymphoid) but mostly large (monocytoid). These large peritoneal macrophages have not been studied extensively here or elsewhere. Since they are thought to be related to alveolar phagocytes, physiological studies of these cells are contemplated.

Auto-agglutination. The tendency of exudate cells in suspension to clump together has often been noted. This phenomenon constitutes a major obstacle to any study requiring a uniform cell suspension. Many experimentalists have attempted with little success to devise procedures which minimize this auto-agglutination. The phenomenon is of sufficient practical and theoretical importance to deserve study, and a few pertinent observations (with the polymorphonuclear exudate cells) have been made in this laboratory.

This initial production of a completely unclumped suspension has not been satisfactorily accomplished. If the original cell sediment (obtained by slow centrifugation) is resuspended by gentle shaking in iced 0.9% NaCl (with or without 20% by volume of isotonic phosphate buffer at pH 6.0), most of the cells are

dispersed but a few microscopic clumps remain. Heparin (1 mg/100 ml) and gelatin (0.1%) may be helpful in depressing the stickiness of these cells, but sodium hexametaphosphate (5×10^{-3} M) appears to be superior. It seems possible that the latter is effective by virtue of its ability to complex Ca^{++} which may be present at the cell surface or in the supernatant in trace amounts. Its protective action against clumping has not yet been compared with sodium oxalate or citrate, but it has proved to be non-toxic to the cells at this concentration, and it is neither metabolized nor hydrolyzed by them (during a 3-hour incubation at 37° C). Of these anti-clumping measures, however, a low temperature (iced solutions) appears to be the one most efficacious.

Samples of the original cell suspension (unclumped) are added to various test-solutions, and these mixtures are incubated at 37° C in small glass vials which are slowly (8 rpm) rotated end over end to prevent sedimentation. Periodically, attempts are made to estimate the extent of the clumping process by one of the following means: 1. visual estimations of the relative amount of macroscopic clumping; 2. mioroscopic counts of the concentration of unclumped cells; 3. turbidity readings on the supernatant after a short preliminary results have been disappointing because the clumping process has proven to be so capricious.

Plasma and serum (with added PO_4 buffer) consistently accelerate the auto-agglutination of these cells. The same is true of a mixture of KCl, $CaCl_2$, and $MgSO_4$ when added in the usual physiological proportions to cells suspended in buffered NaCl (0.9%). It is suspected that the Ca^{++} is the most active agent in these mixtures. In 0.9% NaCl diluted 4 to 1 with isotonic phosphate buffers with pH's ranging from 5.5 to 8.5, the cells clump most completely (though not always fastest) the higher the pH. At high pH's, clumps are large and very tenacious; they cannot be disrupted by any means compatible with cell survival. At low pH's,

clumps are small and so friable that they are disrupted by procedures designed to measure them. Cells in clumps formed at pH 7.4 cannot be satisfactorily dispersed by mechanical stirring or by other means which have been tested. A few attempts to study the kinetics of the clumping process by turbidity measurements have yielded data suggesting that three successive phases occur; an initial slow phase, a rapid phase and a final slow phase, the latter in spite of the fact that appreciable concentrations of unclumped cells are generally still present.

It is apparent that these preliminary observations are not subject to any readily available explanation or interpretation. Many of these observations are reminiscent of those of Loeb (Alexander's Colloid Chem., 2, 487, 1928) on the agglutination of Limulus amebocytes. Some similarities to the problem of platelet agglutination are evident. The data demonstrate that the clumping of these exudate cells is not dictated by the relatively simple physicochemical influences which determine the agglutination of bacterial suspensions or the stability of dust particles in aqueous solutions.

Survival of Exudate Cells in Vitro. Polymorphonuclear cells obtained from the rabbit peritoneal cavity can be kept alive in vitro for a considerable time. When suspended in equal parts of 0.15 M NaCl (0.9%) and 0.1 M sodium acid phosphates (pH 7.4) and kept in a refrigerator (about 5° C), 50% of the cells are alive after 1 week. In unbuffered saline (pH 5-6), 50% of the cells are dead in 3 to 4 days, and a similar mortality at this pH is seen in unbuffered Ringer solution. Bacterial contamination complicates such experiments, but recent trials indicate that this problem can be minimized by the addition of antibiotics, a measure which here appears to be a satisfactory substitute for the tedious techniques necessary in maintaining complete sterility.

In these studies, values of mortality represent that percentage of cells

in any sample which stains with eosin Y (1:1000 in buffered isotonic saline) in a wet mount. This procedure has proven very satisfactory in that staining is almost instantaneous and any one cell is either intensely stained or not at all. From the literature, however, it appears that this criterion has not been adequately compared with other indices of cell death. The major inadequacy of the procedure, however, is that clumped cells cannot be adequately counted. In practice the mortality percentages refer to those cells which in any suspension remain unclumped or can be unclumped by gentle stirring. Whether these cells are representative of the total population has not been determined. In hopes of clarifying this question, a fluorophotometric analysis for eosin has been devised, but a quantitative study on eosin-uptake by cells has not proven successful.

Polymorphonuclear exudate cells suspended in 0.9% NaCl (diluted 3 to 1 with isotonic phosphate buffer at pH 7.4) may be incubated at 37° C for 90 minutes without a detectable rise in mortality. In this medium, the following drugs at the specified concentrations have no detectable lethal action under these circumstances; colchicine, 0.32 mg/ml, mapharsen, 3 mg/ml, sodium salicylate, 10 mg/ml, sodium sulfathiozole, 5 mg/ml, dihydrostreptomysin sulfate, 25 mg/ml, and penicillin G, 0.6 mg/ml. In contrast the following solutions (buffered to pH 7.4) kill 50% of the cells in a 90-minute incubation; pontocaine, 0.2 mg/ml, procaine, 20 mg/ml and quinine dihydrochloride, 0.2 mg/ml.

Experiments now in progress suggest that the phagocytosis of crushed quartz (4 to 6 microns in diameter) may slightly reduce a cell's life expectancy in vitro. The effect is not large and may prove to be within experimental error. If it proves to be a valid observation, its significance can be appraised only after similar experiments are completed with other substances (e.g., carbon, BeO, UO_2).

Summary. Preliminary observations on the auto-agglutination and in vitro survival of rabbit phagocytes are described.

Problem Code: Be.3 (Toxic Limits)

Section Code: 3230

Author: E. A. Maynard

Radiographs taken of rats 5 months to 15 months following a single injection of either 2000 or 3000 milligrams of Be Oxide (fluorescent grade) per kilogram of body weight discloses a peculiar condition in the long bone. This abnormality has the appearance of hypercalcification. Actually it may prove upon histopathological examination to be the result of a failure to resorb calcium as bone growth proceeds. Ten of eleven animals injected intraperitoneally and examined radiographically under the condition described above show bone lesions.

A bone lesion of similar nature has been discovered radiographically in rats which have been fed high dietary percentages of beryllium sulfate over a period of more than one month.

Problem Code: Be.5 (Mechanism of Toxic Effect)

Section Code: 3210

Author: C. J. Spiegl

Changes in Blood Lipid Ratios as an Index of Beryllium Poisoning.

In several experiments in which beryllium poisoning was induced in dogs, either by inhalation or by injection, changes were noted in the lipids of the red blood cell. In particular, the ratio of phospholipid to free cholesterol

increased, although only to the extent of approximately two standard deviations from the normal. Consequently, it was of interest to investigate this criterion of toxicity somewhat further. The current experiment was designed to follow the red cell lipid concentrations for at least 120 days, the approximate life-span of the dog erythrocyte, and also to determine the effect of previous beryllium administration.

One dog, injected one year previously with 0.08 mg Be/kg as $BeSO_4.4H_2O$ was injected intravenously with 0.25 mg Be/kg as $BeSO_4.4H_2O$. Another dog, normal in all respects and previously untreated, was injected with 0.10 mg Be/kg. Blood samples were taken at intervals indicated in Table 1 below and the red cells were analyzed for phospholipid and free cholesterol according to methods previously outlined.

TABLE 1

RATIO OF PHOSPHOLIPID TO FREE CHOLESTEROL IN RED BLOOD CELLS OF DOGS INJECTED WITH $BeSO_4.4H_2O$

Time After Injection	Dog Previously Given Be	Dog Previously Normal
weeks		
Control	2.44	2.53
1	2.62	2.56
5	2.47	2.52
10	3.03	2.74
11	2.90	2.84
12	2.50	2.49
16	2.52	2.66
22	2.42	2.39

The results obtained are shown in Table 1. In neither dog was any significant early rise in the ratio of phospholipid to free cholesterol observed. During the 10th and 11th weeks after injection, however, both dogs showed lipid ratios considerably above the normal range. After the 12th week, all values were

[illegible] normal.

The finding that the phospholipid to free cholesterol ratio in red cells is definitely abnormal during the 10th and 11th weeks after administration of beryllium suggests, 1. that this criterion may be of value during a certain phase of beryllium poisoning and also 2. that the effect of beryllium is not directly upon the red cell but rather upon the hemopoietic system. The time of appearance of the lipid changes coincides with the period at which most pronounced anemia occurred in dogs following inhalation of beryllium sulfate mist.

The results indicate also that a previous administration of beryllium affects the rise in phospholipid to free cholesterol ratio in the red blood cell of the dog only slightly, if at all, and that the difference between injection of 0.25 mg Be/kg and 0.10 mg Be/kg as $BeSO_4.4H_2O$ is small.

Problem Code: Be.6 (Methods of Detection of Poisoning, Prophylaxis, Treatment and Protection)

Section Code: 3210

Author: H. E. Stokinger

Beryllium Content of Tissues Following Recovery from Acute Experimental Beryllium Poisoning.

Much discussion has centered on the presence or absence of beryllium in tissues in relation to the disease processes. One aspect of this has now been investigated, namely, the search for beryllium in body tissues following recovery from acute experimental poisoning. Four dogs were exposed to beryllium sulfate mist for the 3-fold purpose of determing: whether recovery occurs from the toxic anemia produced by the inhalation of beryllium salts, and if so; the time required;

and whether recovery is compatible with the presence of beryllium in the tissues. If the latter is established, it would indicate that a tissue tolerance to beryllium occurs, in animals at least, and the finding might serve as a basis for the explanation why some individuals appear resistant while others are susceptible to beryllium, the lack of the ability to develop a tolerance thus leading to manifestations of beryllium poisoning. Anemia was used as a criterion of recovery because it has been shown to be a sensitive indicator of acute beryllium poisoning in dogs (1).

The plan of the experiment was simple. Four growing dogs, weight range from 5.6-9.7 kg were exposed to beryllium sulfate mist at a concentration of 3.6 mg/m^3 of air, 6 hours daily until a toxic anemia, resembling a macrocytic type, developed. Anemia was considered established when a decrease of 2 million red blood cells per mm^3 and at least a decrease of 3 g% in hemoglobin concentration occurred. Upon attainment of the anemia, the animals were removed from the exposure to beryllium and placed on their regular diet of dog chow supplemented with Evr Redy bone meal for observation of recovery. During this period, as well as during and prior to exposure for 3 weeks, red blood counts, hemoglobin concentration and hematocrit were determined weekly. Upon recovery, animals were sacrificed and the tissues analyzed for beryllium content spectrographically by methods developed by Steadman (2). The samples of femur for analysis comprised the head and one inch of the shaft.

Results. Table 1 (Page 75) presents the data on the change in red blood count and hemoglobin concentration in the dogs as the result of exposure. Simultaneously, with the production of anemia there was a marked loss in weight of 2 of the dogs (one of which died) and no appreciable change in the other two. The dying dog lost 2.2 kg in 34 days and dog 1418 lost 3 kg in 65 days of

TABLE 1

Be CONTENT OF TISSUES AT TIME OF RECOVERY FROM ANEMIA FOLLOWING INHALATION OF BERYLLIUM SULFATE

Dog No.	Time of Exposure Before Removal from Chamber	Time of Development of Anemia (a)	Degree of Anemia Decrease in		Time of Recovery From Anemia	Time From End of Recovery to Sacrifice	Be Content of Tissues Micrograms per gram fresh tissue			
			RBC	Hb			Lung	Bone	Liver	Kidney
	days	days	$x10^6$	g%	days	days				
1432	70	66	2.0	6.6	104	0	2.9	1.3	0.5	0.1
1418	65	70	2.4	7.4	115	21	1.2	1.2	0.4	0.02
1425	64	55	1.7-	3.4	115	21	0.5	0.4	0.1	0.002

(a) Time of greatest decrease in RBC count and hemoglobin concentration.

exposure. Recovery from anemia was associated in every instance either with complete weight recovery (dog 1418) or a weight gain of 1.2 kg in each of the 2 dogs, 1425 and 1432.

It is seen in Table 1 (Page 75) that anemia developed after about 2 months of daily exposure (55-70 days) and that 104-115 days was required to restore a normal blood picture (6.6-7 million red blood cells per mm^3, 14-16.3 g% hemoglobin). At sacrifice of the dogs upon recovery, beryllium was found in appreciable quantities in all 4 tissues analyzed, lung, femur, liver and kidney Table 1 (Page 75).

Thus has been demonstrated: 1. that recovery occurs from the toxic anemia produced by inhalation of beryllium salts; 2. that this recovery requires, on a normal diet, a period of from 3-4 months, and further 3. the recovery occurs despite relatively large quantities of beryllium in the bone of the order of 1 $\mu g/g$. At the time of recovery from anemia, the animals appeared normal both in respect to weight gain and appearance.

Whether recovery from the anemia occurred because growth of the bone removed beryllium from more active blood-forming elements, the probable site of original deposition, or whether beryllium later entered into a physiologically inactive type of crystal lattice remain points to be ascertained.

Bibliography

1. Stokinger, H. E., et al., A.E.C. declassified document UR-84

2. Steadman, L. T., AEC. declassified document UR-18

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PROGRAM S.M.

SPECIAL MATERIALS

Problem Code: S.M.3 (Toxic Limits)

Section Code: 3210

Authors: S. Laskin, P. Frank, D. Meier, R. Wilson

Efficiency of Sampling Methods for the Collection of Toxic Atmospheric Impurities.

Studies have continued on the investigation of methods for the determination of the absolute efficiency of air sampling devices. For the present, emphasis has been placed on the filter paper dust sampler and on the analysis of the factors determining its efficiency. The data to date have indicated an extremely complex problem with such variables as sampling velocity, particle size, relative humidity and the physical nature of the particular aerosol being intimately related to the sampling efficiency. Although aerosol concentration levels were previously reported not to have large effects on sampling efficiency (about 1-2%), more complete results have indicated that this variable is of some significance in the proper analysis of the effect of sampling velocity. In order to isolate the variables, the current studies have been limited to the effects of concentration and sampling velocity on the efficiency of Whatman #41 filter paper. The studies reported below required large numbers of samples to show statistically significant values. Since all analyses have not been completed, the results reported are of a preliminary nature and are presented to indicate the direction and progress of the work.

Fixed conditions were maintained with sodium chloride aerosols dispersed and controlled as described in the previous Quarterly Report (UR-87). Concentrations were analyzed by means of the flame photometer. Humidity was controlled

to a range of 75-88% to simulate a wet aerosol. This condition as indicated previously resulted in lower absolute efficiencies than obtained with dry aerosols and permitted a wide exploration of concentration and sampling velocity variables. The average particle-size mass median was 1.03 μ with a corresponding geometric standard deviation of 1.60. To minimize the possible variation in filter paper porosity, discs used in each experiment were cut from a single sheet of paper.

In order to explore the full range of sampling velocities from 5 to above 30 liters per minute, it was necessary to re-investigate the possibilities of a filter paper train as a method for determining absolute efficiency. The liquid nitrogen trap and the flame photometer were found to be unsatisfactory at higher velocities. In addition, the large number of samples required indicated the need for a more practical field type of instrument. A multiple sampler was developed permitting the arrangement in series of sampling papers up to as many as 12 units. Construction details will be described in a later report.

For the first studies samples were collected from a sodium chloride aerosol atmosphere at an average concentration of 60 mg/m^3. The average sampling velocity was 12 liters/min. Samples were first collected on single filter papers with the air exhausting to the flame photometer to determine losses. This was repeated with increasing numbers of papers in a train until the flame photometer readings indicated a negligible concentration.

Table 1 (Page 79) shows the results obtained with a series up to 6 filter papers in a train. The concentrations passing a single filter paper decreased from 2.84 mg/m^3 to 0.03 mg/m^3 for the series of 6 papers. Since the latter value represented only 1% of the concentration passing the first filter paper, it was considered negligible. The efficiencies of the papers in the series are given as a percentage of total concentration collected. These show a rapid increase

TABLE 1

EFFICIENCIES OF THE ELEMENTS OF A SAMPLING TRAIN OF WHATMAN #41 FILTER PAPER FOR A SODIUM CHLORIDE AEROSOL ATMOSPHERE*

Cumulative No. of Filter Papers in Train	Concentration Collected by Filter Paper Train	Concentration(A) Passing Train	Total Collected by Filter Paper Train
	mg $NaCl/m^3$	mg $NaCl/m^3$	%
1	56.80	2.84	95.24
2	58.82	0.82	98.63
3	59.45	0.29	99.68
4	59.54	0.10	99.85
5	59.57	0.07	99.88
6	59.61	0.03	99.95

* Average air concentration of 60 mg/m^3 at a sampling rate of 12 liters/min.

(A) Analysis by flame photometer

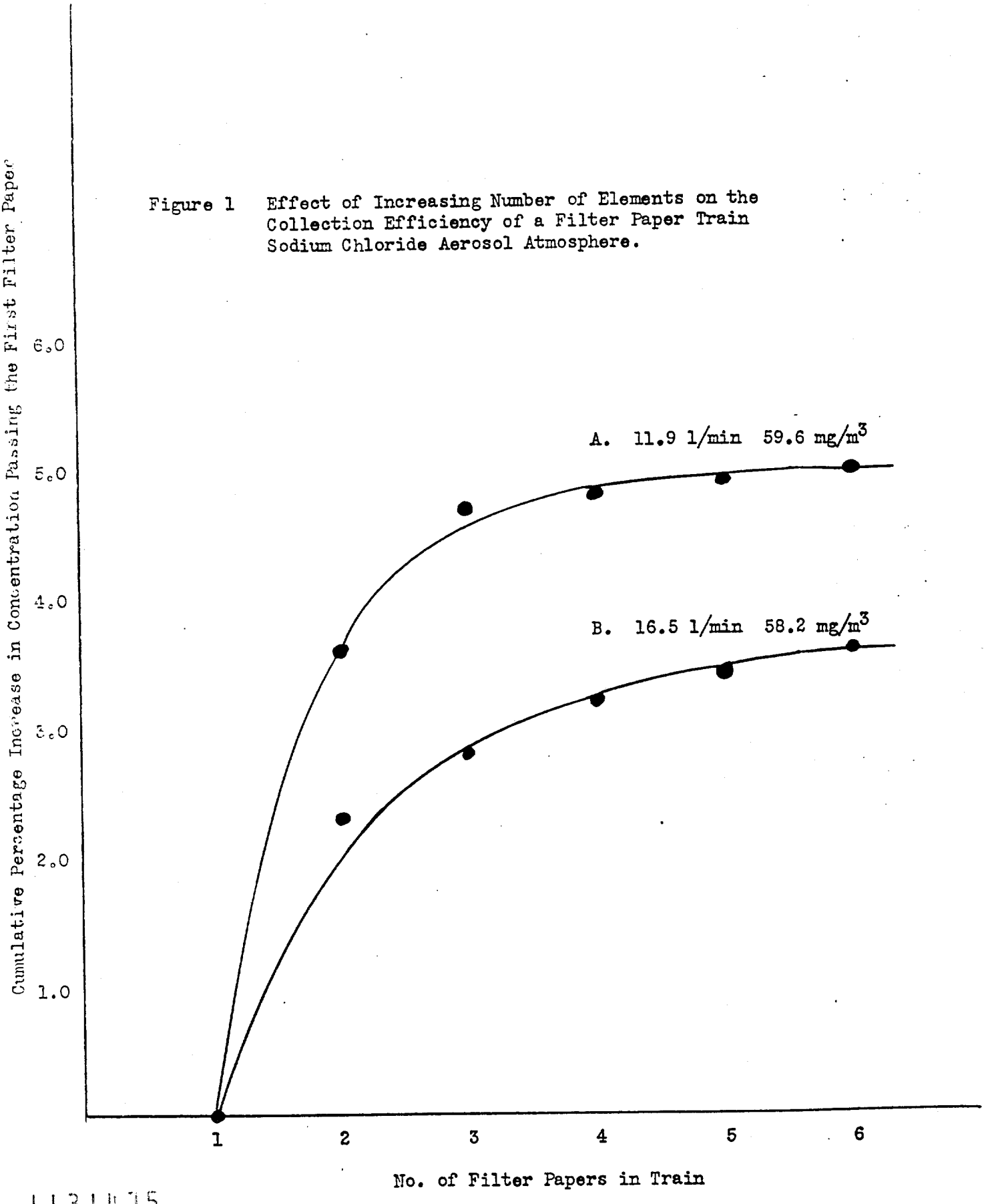
from 95.24% for one paper to 99.95% for the train of six.

Figure 1 (Page 81), Curve A, shows a plot of the same data recalculated by neglecting the six-paper train loss to the flame photometer. The concentrations collected on each of the papers is expressed in terms of cumulative percentage increase above the value of the first paper, similar to the results of Table 1 (Page 79). The curve illustrates the increase in efficiency with increasing elements of the filter paper train. Above four papers, the curve approaches a limiting value of 5% asymptotically. In comparison with the value of 4.8% obtained from the flame photometer analysis of the material passing the first filter paper, excellent agreement was obtained. This permitted the application of the graphical procedure to the interpretation of samples collected by multiple filter paper trains at sampling velocities above the limits of the flame photometer method.

Curve B, Figure 1 (Page 81), shows a plot of the data obtained at a higher sampling velocity (16.5 liters/min.). A six-filter-paper train was used here also to collect the sample and analyses of each element of the train resulted in a 96.4% efficiency obtained for the first paper. The parallelism of the curves appeared to justify the procedure. On the basis of similar studies, it was temporarily assumed that for exploratory experiments, a six-paper train would yield results that closely approached the absolute efficiency of the flame photometer. One other factor of interest apparent in Figure 1 is the increased efficiency obtained with increased sampling velocity in agreement with other results reported below.

Several exploratory studies of efficiency have been made with a six-filter-paper train 1. over a concentration range of from 13 to 59 mg NaCl/m^3 and 2. with sampling velocities from 7.1 to 31.1 liters/min. Typical results

Figure 1 Effect of Increasing Number of Elements on the Collection Efficiency of a Filter Paper Train Sodium Chloride Aerosol Atmosphere.



obtained in one study are given in Table 2 below for a 58 mg/m3 sodium chloride aerosol atmosphere sampled at an average rate of 7.4 liters/min. Concentrations obtained varied from 45 to 62 mg/m^3 and velocities from 6.7 to 7.8 liters/min. Efficiencies are expressed as the percentage of the total aerosol that was retained by the first filter paper. An average value of 93.0% with a range in values from 92.0 to 94.0% were obtained.

TABLE 2

EFFICIENCY OF WHATMAN #41 FILTER PAPERS IN SAMPLING SODIUM CHLORIDE ATMOSPHERES, AVERAGE CONCENTRATION 58 mg NaCl/m^3 AND WITH AVERAGE SAMPLING VELOCITY OF 7.4 LITERS/MINUTE

Sample No.	Air Concentration	Sampling Velocity	Amount Retained by First Filter Paper
	mg NaCl/m3	l/min	%
1	44.8	7.58	92.2
2	60.0	6.74	93.3
3	59.0	7.50	93.2
4	57.1	7.31	93.8
5	61.0	7.00	92.0
6	60.5	7.82	92.6
7	59.5	7.57	94.0
8	59.7	7.75	93.8
9	61.8	7.53	92.8
10	61.0	7.41	92.0

Table 3 below shows the summarized results of the studies completed to date on the efficiency of one Whatman #41 filter paper circle as a function of sampling velocity and concentration, and as determined by the filter-paper train method. At concentration levels similar to those reported in Table 2 (Page 82) but with increased sampling velocity, the efficiency increased from a value of 93.0% at 7.4 liters/min to 97.0% at 16.5 liters/min.

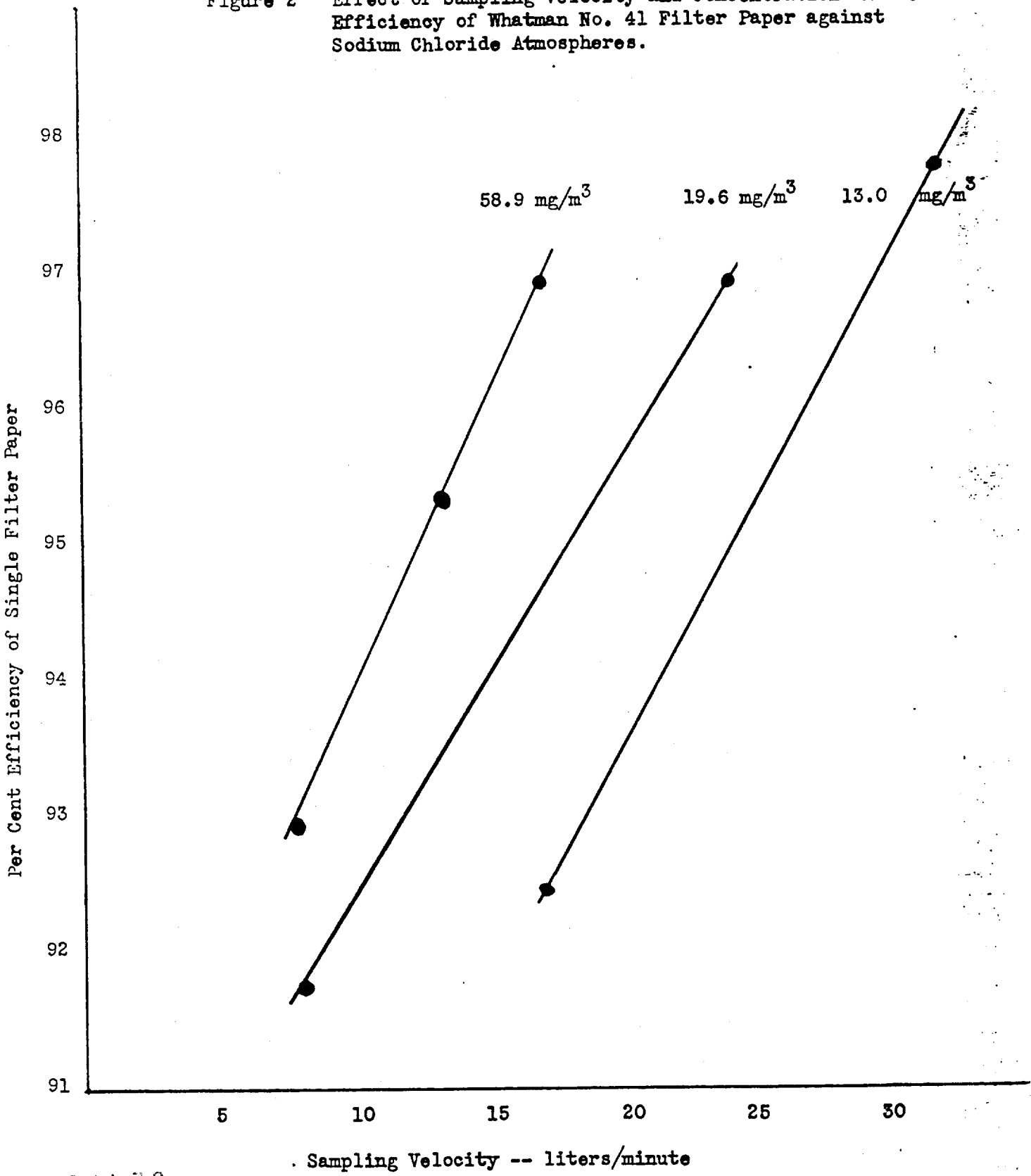
TABLE 3

EFFICIENCY OF WHATMAN #41 FILTER PAPER AS A FUNCTION OF SAMPLING VELOCITY AND CONCENTRATION OF SODIUM CHLORIDE AEROSOL ATMOSPHERES

Exp. No.	No. of Samples	Concentration		Sampling Velocity		% Retained by First Filter Paper	
		Mean	Range	Mean	Range	Mean	Range
		mg NaCl/m^3		liters/min			
1	10	58.4	44.8-61.8	7.4	6.7-7.4	93.0	92.2-94.0
2	10	59.2	55.5-63.0	12.7	12.1-13.3	95.4	95.0-96.2
3	10	59.0	47.3-64.5	16.5	16.0-16.9	97.0	96.0-98.0
4	4	19.2	18.2-20.3	7.6	7.3-7.9	91.8	89.3-93.1
5	9	20.0	18.6-21.0	23.3	22.2-24.3	97.0	96.2-97.8
6	9	13.0	11.5-13.7	16.6	16.2-16.8	92.5	91.7-93.5
7	9	13.0	12.5-13.5	31.1	28.0-33.3	97.6	97.1-98.0

Similar data are shown for concentration ranges at 13 and 20 mg/m^3. The mean results, graphically illustrated in Figure 2 (Page 84), indicate a linear increase in efficiency of Whatman #41 filter paper with increase in sampling velocity over the range explored. In addition, efficiencies were found to decrease with decrease in concentration. The linearity of the curves and

Figure 2 Effect of Sampling Velocity and Concentration on the Efficiency of Whatman No. 41 Filter Paper against Sodium Chloride Atmospheres.



approximate parallelism suggest the possibility that with more complete data a mathematical relationship expressing efficiency as a function of concentration and sampling velocity may be developed. Further work is in progress on the accumulation of the required data.

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PROGRAM I.S.

ISOTOPES

Problem Code: I.S.1 (Tracer Chemistry)

Section Code: 3120

Authors: L. L. Miller, W. F. Bale, C. G. Bly and M. Watson

The availability of lysine-ϵ-C^{14} affords us a tracer with which the production or turnover of the plasma protein fractions can be followed, even when the conditions of a steady state would preclude detection of change by conventional chemical methods.

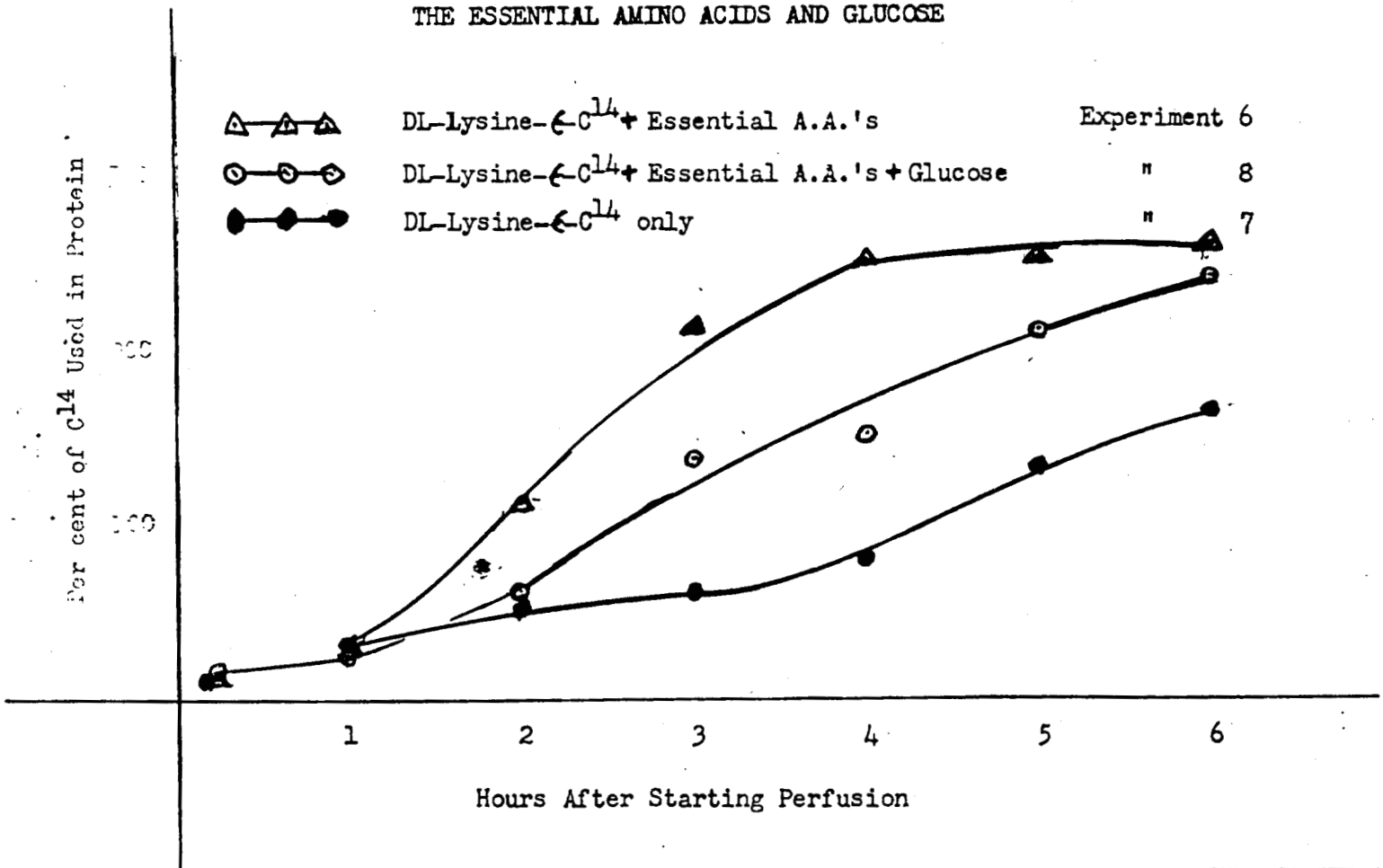
Method: A continuous perfusion system consisting of a reservoir, a pump, an oxygenator, and a coarse filter is filled with whole heparinized rat blood which has been diluted with physiological saline. An adult rat is then anesthetized with ether and the liver rapidly removed in toto after cannulas have been placed in the portal vein and the superior vena cava. Usually not more than 15 minutes time elapsed between the interruption of the hepatic blood flow and the start of active perfusion with oxygenated blood. (The blood is obtained shortly before use by cardiac puncture of rat donors. Heparin is used as anticoagulant, and the blood is diluted with one-third its volume of physiological saline so that the final red cell hematocrit is 25 to 30 volumes per cent.) The DL-lysine-ϵ-C^{14}, the glucose, and the mixture of essential amino acids plus glycine (Mixture VUJ-N, Lot 6R6149, Merck and Co. Inc.) (3) were added to the blood as solutions in a small volume of water. In most experiments the blood and added amino acids were circulated to effect mixing for 10 or 15 minutes prior to removal of the first sample of blood. Other samples were removed at one hour intervals for 6 hours. The methods for determing C^{14} activity total plasma protein, albumin and globulin fractions, and fibrinogen are those previously described (4).

Results: The results of several experiments are graphically presented in Figure 1 (Page 88). The final (sixth hour) data are further compared in Table 1 (Page 89).

It is at once clear that the isolated surviving rat liver perfused with

THE EFFECT OF SUPPLEMENTING LYSINE WITH THE ESSENTIAL AMINO ACIDS AND GLUCOSE

FIGURE 1



COMPARISON OF C^{14} INCORPORATION IN ALBUMIN AND GLOBULIN FRACTIONS

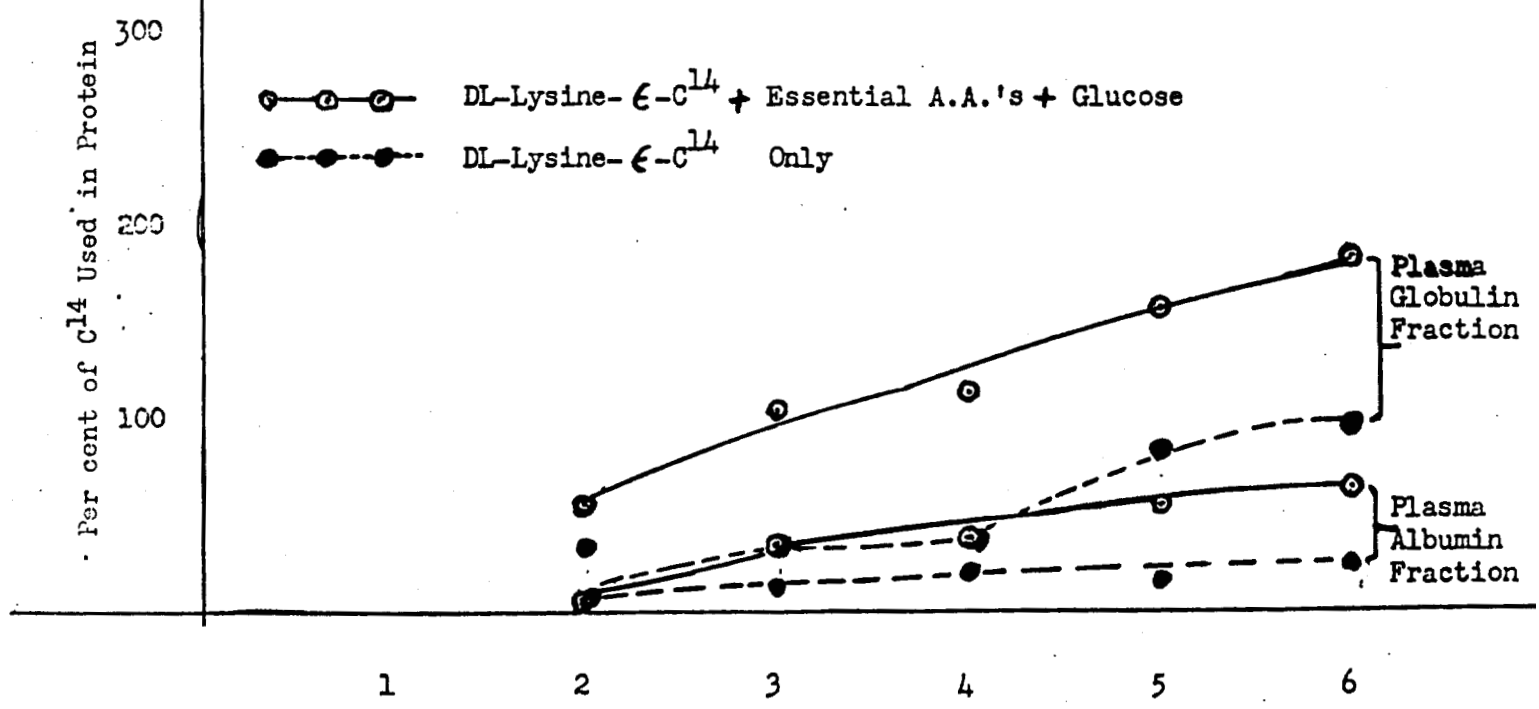


TABLE 1

THE INCORPORATION OF C^{14}-LABELED LYSINE IN PLASMA AND LIVER PROTEINS

	Lysine Only		Lysine and Essential Amino Acids			Lysine and Essential Amino Acids & Glucose		
Dose of C^{14} in Arbitrary Units	Exp. 4	Exp. 7	Exp. 2	Exp. 8	Exp. 10	Exp. 3	Exp. 6	Exp. 9
	66.6	133	66.6	133	304	66.6	133	152
% Dose in Total Plasma Protein	1.89	1.25	7.56	2.61	2.30	5.34	3.44	3.70
% Dose in Total Plasma Albumin	0.47	0.27	1.26	1.11	0.76	1.17	0.63	1.32
% Dose Per Gram Albumin Carbon	0.75	0.77	2.38	1.54	1.14	1.30	0.80	1.24
% Due in Total Plasma Globulin	1.42	0.98	6.30	1.50	1.54	4.17	1.81	2.38
% Dose Per Gram Globulin Carbon	1.58	1.47	5.18	1.56	1.62	3.28	1.27	2.06
% Dose in Total Liver Protein	3.48	1.45	5.21	2.21		4.58	3.51	3.79
% Dose Per Gram Liver (Dry Weight)	1.44	0.69	2.09	1.27		1.89	2.10	1.97
% Dose Per Gram Fibrinogen		1.39		1.86		3.33	2.98	1.16
% Dose Per Gram Fibrinogen Carbon		2.35		3.27		5.84	5.24	2.04

oxygenated blood is capable of incorporating the C^{14} of a labeled amino acid into proteins, some of which appear in the blood and are indistinguishable from plasma proteins, viz., fibrinogen and the albumin and globulin fractions. Furthermore, the addition of the amino acids essential for plasma protein production greatly enhances the extent of incorporation in the plasma and liver proteins. The effect of added glucose is not clear from the experiment cited, although in some of the experiments (see Table 1, Page 89) the addition of glucose at least appears to favor greater incorporation of the isotope in the liver protein and in fibrinogen. The presence of glucose in the blood used for the perfusion and an undetermined amount of glycogen in the livers may conceal a real effect of glucose. The glucose was added in some of the experiments to ensure the presence of an energy yielding substrate for the process of protein synthesis which requires considerable energy.

Another noteworthy finding which is apparent from Figure 1 (Page 88) and repeatedly observed in all of the experiments is seen in the greater incorporation of the isotopic label in the globulin fraction as contrasted to the albumin fraction. This is of interest because it is qualitatively identical and quantitatively very similar to the distribution of the isotope in the plasma proteins of normal dogs after the feeding of single doses of C^{14} labeled lysine (4). This may well be regarded as evidence in favor of the view that the isolated surviving liver perfused with whole blood is responding in an essentially physiological manner.

Discussion: It is possible to estimate the number of milligrams of plasma protein and liver protein produced per gram of dry liver protein in each experiment. The estimates shown in Table 1 (Page 89) are based on the assumption; that the C^{14} is incorporated in the protein as L-lysine only (5),

and that the proteins contain an average of 6.25% lysine. The first assumption may not be entirely valid since in the dog (6, 7) significant though small amounts of the C^{14} fed as lysine were found in glutamic acid, aspartic acid, and arginine.

It is at once clear that without the other essential amino acids, lysine is incorporated into very little of either liver or plasma proteins. The small yield observed may be referable to the supplemental action of the small amounts of free amino acids in both liver and blood.

The supplemental addition of the essential amino acids to lysine results in a ten to twenty fold greater incorporation of the isotope in the proteins. This probably represents true protein production since, if the labeled lysine molecules merely exchanged with unlabeled lysine residues in already existent protein structures (8), it seems reasonable to expect that such exchange would occur as well with lysine alone as when the other essential amino acids are present. The occurrence of true protein synthesis is a more likely explanation, especially since the above effect of amino acid supplementation parallels the results obtained by others in the intact dog (9) and rat (10) in studies of plasma protein production.

If the milligrams of plasma protein synthesized per gram of dry liver may be used as a criterion of the effectiveness of experimental conditions, the addition of extra glucose to the experimental system is without favorable effect (Table 2, Page 92). In fact, the largest protein productions are seen in Experiment 2 and 10, to which no extra glucose was added. These experiments differ from the others in the fact that the perfusion pressure (and hence the perfusion rate) was 1.5 to 2 times the perfusion pressure used in Experiments 3, 6, and 9. This suggests that, given ample substrate, the higher mean oxygen tension of the

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TABLE 2

THE EFFECT OF THE ESSENTIAL AMINO ACIDS ON LIVER AND PLASMA PROTEIN PRODUCTION

	Lysine Only		Lysine and Essential Amino Acids			Lysine and Essential Amino Acids and Glucose		
	Exp. 4.	Exp. 7	Exp. 2	Exp. 8	Exp. 10	Exp. 3	Exp. 6	Exp. 9
Mgs. Plasma Protein Produced Per Gram Liver (DW)*	0.11	0.17	2.22	1.42	2.20	1.66	1.04	1.26
Mgs. Liver Protein Produced Per Gram Liver (DW)	0.20	0.20	0.66	1.20		0.88	0.90	1.29
Total Mgs. Protein Produced Per Gram Liver (DW)	0.31	0.37	2.88	2.62	2.20+	2.54	1.94	2.54
Perfusion Rate Drops Per Minute	150.	60-150		180-250	200-380		130-200	150-240
Perfusion Pressure in Cm. Blood	14.	15-16	25-30	14-16	25.	13-15	14.	15-17
Total L-Lysine Present Mgs.	0.436	0.872	2.491	2.544	6.030	2.491	2.952	3.065
**Total Amino Acids Added as VUJ-N Mixture Mgs.			19.	20.	40.	19.	20.	20.

*Dry weight

**The composition of the VUJ-N mixture is as follows:

l(+)-Arginine HCl	8.0
l(+)-Histidine HCl-H_2O	4.0
l(+)-Isoleucine	7.8
l(+)-Leucine	17.5
l(+)-Lysine HCl	12.3
l(-)-Methionine	5.5
dl-Methionine	1.2
l(-)-Phenylalanine	8.7
l(-)-Threonine	1.7
dl-Threonine	7.4
dl-Tryptophane	1.8
l(+)-Valine	6.1
Glycine	10.0
	92.0

perfusing blood is the critical factor in the higher rate of plasma protein synthesis. Unfortunately, actual measurements of the oxygen content or tension of the blood entering and leaving the liver preparations were not made in these experiments, but are planned. However, if one considers the sum of the liver and plasma protein produced, the effect of the higher perfusion rate (Experiment 2) is not as marked. It may be that the higher perfusion rate is effective in removing from the liver already formed plasma proteins.

It is of some interest to examine the quantitative relation of the fibrinogen (i.e. fibrin) produced to the total plasma protein and the albumin and globulin fractions. In Experiment 3, for example, with the total of approximately 230 mg. % of fibrinogen, the fibrinogen contains C^{14} equivalent to 0.77% of the total dose of C^{14} used. This corresponds to 14% of the total C^{14} incorporated in the plasma proteins as a whole, whereas the fibrinogen represents but 5.7% of the total plasma protein. Similar estimates for the other experiments show a range of from 1.4 to 6 times as much C^{14} incorporated per gram in the fibrinogen as in the plasma protein taken as a whole.

Another calculation of interest reveals that the per cent of the total amino acids available converted to plasma and liver proteins ranges from about 9 to 25% (disregarding the unnatural D-Lysine). These figures are easily in the physiologic range for the conversion of fed protein or amino acids to liver and plasma proteins in the rat.

Summary. The surviving isolated rat liver perfused with whole oxygenated heparinized rat blood has been used in studies of plasma and liver protein synthesis with the aid of DL-lysine-ϵ-C^{14}.

For the first time direct experimental evidence affirms the role of the rat liver in the synthesis of rat plasma albumin, globulin, and fibrinogen.

The incorporation of the labeled lysine expressed in terms of the milligrams of protein synthesized is small when lysine only is used. Supplementation with a mixture of the essential amino acids greatly enhances the protein synthesis (ten to twenty fold).

The mean oxygen tension of the perfusing blood is apparently a critical factor in the synthesis of plasma protein by the liver.

Bibliography

1. Berryman, G. H., Bollman, J. L., and Mann, F. C., Am. J. Phys., 139, 556 (1943)

2. Tarver, H. and Reinhardt, W. O., J. Biol. Chem., 167, 395 (1947)

3. Silber, R. H., Seeler, A. O., and Howe, E. E., J. Biol. Chem., 164, 639 (1946)

4. Miller, L. L., Bale, W. F., Yuile, C. L., Masters, R. E., Tishkoff, G. H., and Whipple, G. H., J. Exp. Med., 90, 297 (1947)

5. Weissman, N. and Schoenheimer, R., J. Biol. Chem., 140, 779 (1941)

   Ratner, S., Weissman, N., and Schoenheimer, R., J. Biol. Chem., 147, 549 (1943)

6. Miller, L. L. and Bale, W. F., Fed. Proc., 8, 230 (1949)

7. Miller, L. L., Fed. Proc., 3, 229 (1949)

8. Schoenheimer, R., The Dynamic State of Body Constituents, Harvard University Press (1942)

9. Madden, S. C., Carter, J. C., Kattus, A. A., Jr., Miller, L. L. and Whipple, G. H., J. Exp. Med., 77, 277 (1943)

10. Frazier, L. E., Wissler, R. W., Steffee, C. H., Woolridge, R. L., and Cannon, P. R., J. Nutrition, 33, 65 (1947)

UNCLASSIFIED

PROGRAM I.N.

INSTRUMENTATION (SPECTROSCOPY, ELECTRON MICROSCOPY, X-RAY AND NUCLEAR RADIATION DETECTORS, X-RAY DIFFRACTION, ELECTRONICS)

Problem Code: I.N.1 (Research and Development)

Section Code: 3150

Author: L. T. Steadman

The Spectrochemical Determination of Zironium in Biological Materials

Introduction. The sensitivity of detection of zirconium by spectrographic methods has recently been determined in the course of two representative investigations wherein the experimenters were dealing essentially with pure solutions. Fred, Machtrieb, and Tomkins (1) using a copper spark method and a grating spectrograph obtained a sensitivity of 0.05 micrograms for zirconium for the spectrum line 3392.0 A, and as great as 0.01 micrograms for the line 4149.2 A in the second order spectrum. The porous cup electrode method, also a spark method, developed by Feldman (2) has shown a sensitivity of 0.5 micrograms in the electrode with lines 3273.0 and 3496.2 A.

The spectrochemical method described herewith was developed for use with d.c. carbon arc method of excitation, and for application to the routine analysis of zirconium in biological materials. Many of the techniques used follow closely the spectrochemical procedures described by the author for uranium (3) or beryllium (4), and therefore will not be discussed in great detail. The sensitivity obtained is 0.02 micrograms of zirconium on the electrode. With samples possessing very low concentrations of zirconium the element may be isolated to a large extent by an ether extraction procedure thereby yielding fairly adequate sensitivity for the application of the method to measurements

in biological materials such as urine, soft tissues, and bone.

Excitation of the Samples. The sample with the internal standard, usually in 0.1 ml of solution, is placed in the crater of the negative electrode and dried therein. The electrodes are National Carbon Company specially purified graphite rods, $\frac{1}{4}$ inch in diameter with a crater 3/8 inch deep and 3/16 inch in diameter. The arc is burned for 3 minutes with extra oxygen supplied by means of a chimney built around the negative electrode. The positive electrode is a piece of regular grade $\frac{1}{4}$ inch diameter graphite rod. The arc current is 15 amperes and the line potential 130 volts.

The light from the cathode is projected by means of an 8 cm focal length quartz lens and focused on the collimator lens. The slit width is about 10 microns and the height 9 mm. A half-cylinder rotating sector, 9 mm in diameter, is placed immediately in front of the slit. The spectrum is recorded on 35 mm high contrast positive motion picture film using a Bausch and Lomb medium quartz spectrograph. Relative intensities are determined by measurement of the separation of the two extinction points of a spectrum line for zirconium and a line for platinum which is the internal standard. The spectrum lines measured are zirconium 2571.4 A, and platinum 2650.8 A.

The Enhancement of Zirconium. In the process of developing the carbon arc method it was found that the intensity of the zirconium spectrum is appreciably increased by the presence of the alkalies and certain other elements. This finding has three important consequences. First, since biological samples in general contain Na, K Ca, Mg, etc., it is necessary to use a spectroscopic buffer to maintain constant matrix conditions. Secondly, the regular grade spectroscopic electrodes which do not show the presence of zirconium when burned alone prove to contain about 0.2 micrograms when alkali elements are introduced, and therefore

will show zirconium even when blank biological ash is tested. Thirdly, the use of an enhancing agent is thus made possible, which increases the sensitivity of detection of zirconium.

Since the highest purity graphite does not contain detectable zirconium in the presence of an enhancing agent the procedure now in use consists in adding to the electrode crater about 10 mg KCl as a dry powder before the solution sample is introduced. The KCl increases the sensitivity of measurement about a factor of ten and also serves as the spectroscopic buffer.

Attempts thus far to remove the zirconium from the regular grade electrodes by extraction with acids (3), including HF, have not been successful. The only disadvantage in using the high purity electrodes is that they are about ten times as expensive as the regular grade of graphite.

Calibration Curves. The calibration curve has been established by plotting the ratio of the line intensities of zirconium to platinum against the amount of zirconium. The amount of platinum used is 4 micrograms. Since a lesser sensitivity frequently is satisfactory in cases where the amount of zirconium to be measured is large, a second calibration curve also has been tentatively set up for use with the regular grade electrodes. For this the illumination is reduced by placing a wire mesh screen of transmission about 50% over the projection lens and the amount of platinum is increased to 20 micrograms. The form of the curves may readily be seen from the following tables of coordinates. The positive reading in Table 2 for zero zirconium added to the electrode is due to the residual zirconium in the electrode.

TABLE 1

MEASUREMENT WITH HIGHEST SENSITIVITY

Amount of Zirconium (Micrograms)	0.00	0.02	0.05	0.10	0.20
Intensity of Zirconium 2571.4 A / Intensity of Platinum 2650.8 A	0.00	0.55	1.00	1.50	2.00

TABLE 2

MEASUREMENT WITH LESSER SENSITIVITY

Amount of Zirconium (Micrograms)	0.00	0.20	0.50	1.00	2.00
Intensity Zirconium 2567.6 A / Intensity Platinum 2650.8 A	0.40	0.60	0.95	1.30	1.65

The zirconium standard solution was prepared from $ZrCl_4$. The possible effect on the calibration curve of elements other than those usually present in biological samples has not yet been investigated. The zirconium line 2571.4 A is not interfered with by lines of any of the elements likely to be present in biological samples with the possible exception of iron. A measurement of zirconium may be made satisfactorily in the presence of 100 micrograms of iron on the electrode. However, the zirconium sensitivity is greatly reduced when 1 mg. or more of iron is present.

Ashing of Biological Materials. Initial experiments on the ether extraction of zirconium from the ash of biological samples such as urine and bone when the zirconium was added to the materials already dry ashed proved to be successful. However, when the zirconium was added to the sample before it was dry ashed in

the muffle furnace at 500°C, the recovery on extraction was only a few per cent. The loss was shown not to be due to volatilization of the zirconium, since it could be accounted for by a direct measurement on the ash which was prepared part in solution and part in suspension, but was due to the fact that oxides or other compounds of zirconium are formed which are not soluble in the reagents used in the ether extraction procedure.

Attempts to obtain both the biological ash and the zirconium compounds in solution in HCl by means of treatment with H_2SO_4 or HF have not been successful thus far, as might be expected, although the zirconium oxide is soluble in HF. Also it was found that the glaze on porcelain crucibles contains considerable amounts of zirconium so that porcelain should not be used in analytical work on zirconium. Silica dishes may be satisfactory. The biological samples have therefore been prepared by a wet ashing procedure. The digest may be analyzed directly or the zirconium may be concentrated by separation if the zirconium concentration is very low.

Separation of Zirconium by Ether Extraction. Although standard texts give the ether extraction of zirconium chloride as zero per cent, the extraction of zirconium from a concentrated HCl solution by ether is found to be possible with the use of $CaCl_2$ as a salting out agent in a manner similar to the ether extraction of beryllium (4). The amounts of reagents used have been determined somewhat empirically and are still tentative values. The method for a pure solution is as follows: Add zirconium to 3 ml HCl + 1 ml H_2O. Saturate with powdered anhydrous $CaCl_2$ (about 3 grams). Transfer to a separatory funnel and shake with 15 ml of ethyl ether. Three layers form on standing. The bottom layer containing the salt is drawn off and discarded. Transfer the remaining two layers to a beaker, add water and evaporate off the ether. The

partition coefficient of a single extraction is about 95%. This value has been established by the recovery measurements and also by carrying out a second extraction on the salt residue of the bottom layer.

Urine. To 25 ml fresh urine in a 50 ml pyrex beaker add 5 ml concentrated HNO_3. Boil down on the hot plate until the residue becomes foam like but does not char. Add 2 ml of H_2O + 1 ml HCl. Warm and bring residue into solution. Add 3 ml HCl. Saturate with $CaCl_2$ and proceed as above.

Bone. Digest 2 grams or less of bone in 1 : 1 HNO_3 on the hot plate and evaporate to dryness without charring. Add 3 ml H_2O + 1 ml HCl. Warm and bring residue into solution. Add 5 ml HCl. Saturate with $CaCl_2$ and proceed as above.

Soft Tissues. Digest 10 grams or less of soft tissue in 1:1 HNO_3 with heating. Cool and remove the fat layer. Continue the wet ashing to obtain a white ash by repeated use of HNO_3 and a few ml of $HClO_4$. Treat the ash with hot HCl to insure solution of the zirconium and evaporate to dryness. Dissolve in 4 ml HCl + 2 ml H_2O and proceed as above. If necessary, a partial separation of the zirconium from iron in the extract may be made by evaporating the extract to dryness, dissolving in 3 ml concentrated HCl, and shaking with 30 ml ether. The ether layer will contain 50% of the zirconium but only 10% of the iron.

The methods have been tested thus far in the range 0.1 to 100 micrograms of zirconium. As in the beryllium method, about 10 mg. of calcium salt comes through the extraction so that the method at present does not give a complete isolation of the zirconium, but this amount of salt is not detrimental to the spectrographic measurement of zirconium.

Summary. A spectrographic method using the d.c. carbon arc has been devised for the measurement of zirconium. A sensitivity of 0.02 micrograms on the electrode is obtained by using KCl as an enhancing agent with spectroscopic

buffer. Small amounts of zirconium in biological materials are isolated by ether extraction of the chloride. The sensitivity is about 1 microgram per liter of urine, 0.1 microgram per gram of bone, and 0.001 microgram per gram of soft tissue.

Bibliography

1. Fred, Mark, Nachtrieb, Norman H., and Tomkins, Frank S., J. Opt. Soc. Am., 37, 279-288 (1947)

2. Feldman, Cyrus, Anal. Chem., 21, 1041-6, (1949)

3. Steadman, L. T. AEC Project Report UR-41 (1948)

4. Steadman, L. T., AEC Project Report UR-18 (1948)

Problem Code: I.N.2 (Service)

Section Code: 3150

Author: L. T. Steadman

Spectrographic Service Analyses

1. 280 chamber dust samples were analyzed for beryllium
2. 227 air dust samples were analyzed for beryllium
3. 34 animal tissue samples were analyzed for beryllium
4. 5 human tissue samples were analyzed for beryllium
5. 5 fume samples were analyzed for U, W, and Cu

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EDUCATIONAL PROGRAM

Problem Code: None

Section Code: 3480

Author: J. N. Stannard

The training activities of the Project have increased in scope sufficiently to warrant establishment of an administrative unit to handle pertinent problems. With occupation of the new wing next spring and anticipated increase in student population, an increasing amount of time and energy will perforce be expended on the educational phases of the Project's activities. For this reason a periodic report of developments in the training program will be made.

Fellows in Health Physics, 1948-49. The group of eight AEC Technical Fellows in Health Physics assigned to this facility in October, 1948, completed their training at Rochester during the summer months by assignment to various research projects as technical assistants. The assignments were determined by the aptitude and interests of the trainees. Two assisted with problems in instrumentation, one with the particle size problem, one with radiation chemistry, one with the effects of very short, high intensity x-ray flashes, one with phagocytosis in connection with the beryllium problem, and two with blood changes after x-irradiation.

This group reported by a cooperative arrangement to Brookhaven National Laboratory on September 1st for practical experience with field problems. Under the general direction of Mr. D. Balber, they are receiving experience in area survey, building survey, personnel monitoring, and calibration of instruments. They remain responsible to Rochester until completion of their training on November 1st.

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Three members of the group have received offers for extension of their fellowships to complete research toward a Master's Degree in Radiation Biology. Two are returning to Rochester for this purpose. Two others are entering employment at the National Institutes of Health, U. S. Public Health Service, Bethesda, Maryland, in connection with the operation of a new "hot laboratory" for research in biology and medicine.

Fellows in Radiological Physics, 1949-50. A new group of ten AEC Fellows has just begun training in this field. They have undergraduate degrees in electrical engineering, physics, or related fields, and are able to enter upon more advanced work in instrumentation and radiation measurement than the previous group. Partly for this reason and partly to provide necessary biological background, the group is spending about one-half of its time on biological and medical problems. An outline of the present program and a description of the courses being followed is shown on pages 107-111. Certain alterations have been made for Fellows with advanced training in specific fields, but this outline is generally applicable.

Since a quick review of basic biological principles was needed to qualify the students for physiological studies, a survey course was organized under the general direction of Dr. Elliott Maynard. An outline of this course is shown on pages 112-113. This was an optional program.

Pre- and Post-Doctoral Fellows. In addition to formal training for the Technical Fellowship group, several pre- and post-doctoral fellows supported either by AEC Fellowships or University Fellowships are doing research in the fields of biophysics, pharmacology, and radiation biology. This is being done along conventional academic lines and contributes to the Project's research efforts.

General Comments. The responsible instructors in the courses given

by AEC Project personnel are listed below:

Instrumentation	Herbert Mermagen
Radiation Biology	J. N. Stannard
Industrial Hygiene and Toxicology	Herbert Stokinger and Sid Laskin
Practical Radiological Physics.	Herbert Mermagen and Russell Hayes
Principles of Statistical Inference . . .	Lee Crump

During the period of operation of the first four courses, the training program becomes a major work load for the individual responsible. In addition, many other staff members contribute from a few lectures to several weeks of concentrated activity. It is anticipated that, as the training program expands to include more advanced students, the additional research activities of the student group will compensate somewhat for the time and effort at present needed for the organization and conduct of this new training enterprise.

UNIVERSITY OF ROCHESTER SCHOOL OF MEDICINE AND DENTISTRY

DEPARTMENT OF RADIATION BIOLOGY

OUTLINE OF COURSE IN RADIOLOGICAL PHYSICS FOR 1949-50#

First Semester

Catalogue No.	Title	Credit	Time	Place
Radiation Biology 109	Instrumentation	5 hours	All day Tues. & Thurs. 10:30 A.M. to 12 Noon Oct. 3 to Dec. 2*	Annex and Medical School
Radiation Biology 131	Radiation Biology	5 hours (partly in second semester)	As above Dec. 5 to Feb. 17	Annex, Medical School, and River Campus
Radiation Biology 241	Principles of Statistical Inference	1 hour	Fri. 8:25 A.M.	Medical School
Physics 113	Introduction to Modern Physics	3 hours	Mon., Wed., and Fri. 9:25 A.M. (entire semester)	River Campus
Biology 11	Mammalian Physiology	3 hours (not graduate credit)	Mon. & Fri. afternoons (entire semester)	Medical School
--------	Basic Biology	No credit**	Ten lectures Five Laboratories Sept. 26 to Oct. 7	Annex and Medical School

Being followed by AEC Technical Fellows in Radiological Physics

Second Semester

Catalogue No.	Title	Credit	Time	Place
Pharmacology 210	Industrial Hygiene and Toxicology	5 hours	Same as Rad. Biol. 109 and 131 Feb. 20 to April 28	Annex and Medical School
Radiation Biology 110	Practical Radiological Physics	3 hours	Same as Rad. Biol. 109 May 1 to June 7	Annex and Medical School
Radiation Biology 124	Introduction to Research †	3 hours	Mon. & Fri. afternoons (entire semester)	Annex
Radiation Biology 242	Principles of Statistical Inference	1 hour	Hours to be arranged	Medical School
Physics 114	Nuclear Physics	3 hours	Mon., Wed., Fri. 11:25 A.M. (entire semester)	River Campus

Vacations: As in Medical School Bulletin and June 8 -- July 5 (tentative).

* Part time Oct. 3--10.

** For students lacking biology prerequisit for mammalian physiology.

† May be replaced by research problem for qualified students.

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DESCRIPTION OF COURSES IN RADIOLOGICAL PHYSICS (1949-50)

I. Instrumentation and Practical Radiological Physics

Credit 8 hrs.

This course is given in two sections, one at the outset of study (5 hours credit), the other (3 hours credit) after completion of courses in radiation biology, industrial hygiene and toxicology. The first section is devoted to fundamental electronics, regulated power supply, construction and repair of instrument components, measurement of alpha, beta, and gamma radiation with counters and ionization chambers, and measurement of the international roentgen. The second section consists primarily of application of this information to survey and monitoring problems. In addition the use of air sampling and analysis techniques in combination with radiation measurements is studied to prepare the student for practical problems in evaluating both chemical and radiation hazards from dusty atmospheres. It is contemplated that practical, large-scale survey problems will be presented after completion of this course by transfer of the student to major operating facilities of the U. S. Atomic Energy Commission. Only those students who are not majoring in Physics or Biophysics will be given graduate credit for this course.

II. Radiation Biology

Credit 5 hrs.

A survey is made of the effects of ionizing radiations on living systems and on constituents of body fluids and cells. Emphasis is placed upon changes occurring in mammals and their bearing on the problem of human exposure. The biological basis for radiation tolerance estimates is

discussed in detail, and special problems arising in connection with the entrance of radioactive materials into the body are studied. Genetic effects of radiation are taken up in cooperation with the Department of Biology, College of Arts and Science.

III. Industrial Hygiene and Toxicology

Credit 5 hrs.

A survey course consisting of both lectures and laboratory problems relating to Industrial Hygiene and Toxicology. Topics discussed include the basic principles of toxicology, specific materials constituting industrial hazards, characterization of toxic atmospheres, and the biological applications of statistical methods. The industrial problem is considered from both the protective engineering aspects and the evaluation of hazards in terms of field surveys. Special emphasis is placed on inhalation toxicity, the dust particle-size problem, the materials of interest to the U. S. Atomic Energy Commission. Laboratory problems are designed to give the student experience in the use of various types of sampling and analytical procedures for the evaluation of dust atmospheres and in the use of experimental animals for the determination of maximum allowable concentrations.

IV. Introduction to Research

Credit 3 hrs.

The student is assigned for periods of one month to representative research laboratories within The University of Rochester Atomic Energy Project. By this system of rotation familiarity is gained with various types of research equipment and methods employed by laboratories which the radiological physicist may be required to survey or monitor. No attempt is made to engross the student in details regarding specific research

problems, but a concept of overall objectives and practices is presented.

V. Principles of Statistical Inference

Credit 2 hrs.

The description of frequency distribution. The characteristics of "good" methods of statistical estimation and of testing hypotheses. The relation between statistical methods and experimental research.

VI. Introduction to Modern Physics (given by Department of Physics, College of Arts and Science)

Credit 3 hrs.

A survey of the field of atomic physics with particular emphasis on the general significance of recent developments. The course is intended primarily for students not majoring in Physics.

VII. Introduction to Nuclear Physics (given by Department of Physics, College of Arts and Science)

Credit 3 hrs.

A study of nuclei and nuclear processes with emphasis on new methods and applications. Includes structure of the nucleus, nuclear transformations, fission, methods of producing high energy particles, counting techniques. Applications to the fields of chemistry and biology are emphasized. The subject matter of this course forms a logical continuation of Introduction to Modern Physics.

VIII. Mammalian Physiology (given by Department of Physiology, School of Medicine and Dentistry)

Credit 3 hrs.

An introductory course with emphasis on human physiology. Human as well as other mammalian subjects are used in the laboratory to demonstrate the principles involved. This course is open to all students who have taken Biology 1-2 and Chemistry 1-2. A knowledge of elementary physics is desirable.

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OUTLINE OF BASIC BIOLOGY COURSE*

Lectures:	Instructors:
1. Biology: Scope and relation to other sciences The biological method	Maynard
2. Plant vs. Animal and Kinds of Plants: Comparative characteristics of plants and animals Characteristics of major plant groups	Maynard
3. Kinds of Animals: Classifications based on various characters Characteristics of lower phyla	Maynard
4. Kinds of Animals (cont'd.): Characteristics of higher phyla	Maynard
5. Protoplasm: Physical and chemical nature	Laskin
6. Metabolism: Metabolism and nutrition	Stannard
7. Coordination in the Body: Animal vs. plant Principle of unity in an organism The circulatory system as a coordinating mechanism Endocrine glands	Maynard
8. Coordination in the Body (cont'd): The nervous system as a coordinating mechanism	Blair
9. Cell Reproduction and Inheritance: Mitosis and the gene theory Viruses	Maynard
10. Evolution: Evidences Causes	Maynard

*Given in autumm, 1949, for A.E.C. Radiological Physics Technical Fellows with no undergraduate biology courses.

Laboratory Exercises:	Instructors:
1. Anatomy of the Frog	Maynard, Stannard
2. Anatomy of the Rat	Maynard, Casarett
3. Microorganisms, Use of Microscope	Maynard, Laskin
4. Cell Division	Maynard, Casarett
5. Museum Visit	Maynard, Stannard

Text:

Mavor: GENERAL BIOLOGY

The formal sessions in this course are being followed by informal weekly conferences on specific topics. These will continue until January 1950.

TECHNICAL REPORTS ISSUED FOR DISTRIBUTION

Classified Restricted or Lower

July 1, 1949 thru September 30, 1949

Report No.	Title	Authors	Subject Category
UR-67	"Suggested Maximum Permissible Concentration of Insoluble Uranium Compounds in Air" (RESTRICTED) Issued: 8/2/49	Hodge et al	Health and Biology
UR-76	"The Influence of Infection on the Hematological Effects and Mortality Following Mid-Lethal X-radiation" (UNCLASSIFIED) Issued: 8/18/49	Bennett Rekers et al	Health and Biology
UR-77	"A Preliminary Investigation of Blood Fluoride Levels Following Exposure to Hydrogen Fluoride at a Concentration of Approximately 29 Mg/m^3" (UNCLASSIFIED) Issued: 8/16/49	Smith Gardner	Health and Biology
UR-78	"Hemin Synthesis in Spleen Homogenates" (UNCLASSIFIED) Issued: 8/18/49	Salomon Altman	Health and Biology
UR-79	"The Experimental Study of Flash Burns" (UNCLASSIFIED) Issued: 8/16/49	Payne Hogg Pearse	Health and Biology
UR-80	"The Mechanism of Action of Uranium on Cells" (RESTRICTED) Issued: 8/2/49	Rothstein et al	Health and Biology
UR-81	"Suggested Maximum Allowable Concentrations of Soluble Uranium Compounds in Air" (RESTRICTED) Issued: 8/2/49	Hodge et al	Health and Biology
UR-82	"Urinary Uranium as a Measure of Exposure Hazard" (RESTRICTED) Issued: 7/14/49	Neuman	Health and Biology

Report No.	Title	Authors	Subject Category
UR-84	"The Inhalation Toxicity of Beryllium Sulfate Mist at a Concentration of 1 mg/m^3 for 100 Days" (UNCLASSIFIED) Issued: 9/27/49	Stokinger et al	Health and Biology
UR-86	"Further Polarographic Studies of the Uranyl Citrate Complex" (RESTRICTED) Issued: 9/20/49	Neuman et al	Health and Biology
UR-87	Quarterly Technical Report (RESTRICTED) Issued: 9/15/49		Health and Biology