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Health and Biology

THE UNIVERSITY OF ROCHESTER
Atomic Energy Project
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# QUARTERLY TECHNICAL REPORT

April 1, 1949 thru June 30, 1949



Submitted by: Henry A. Blair
Director

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Health and Biology

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| Hanford Operations Office | 35 |
| Iowa State College | 36 |
| Knolls Atomic Power Laboratory | 37-38 |
| Los Alamos | 39-41 |
| Massachusetts Institute of Technology (Kaufmann) | 42 |
| Mound Laboratory | 43-45 |
| National Advisory Committee for Aeronautics | 46 |
| National Bureau of Standards | 47 |
| Naval Radiological Defense Laboratory | 48 |
| NEPA Project | 49 |
| New Brunswick Laboratory | 50 |
| New York Operations Office | 51-53 |
| North American Aviation, Inc. | 54 |
| Oak Ridge National Laboratory | 55-66 |
| Patent Branch, Washington | 67 |
| Public Health Service | 68 |
| Sandia Laboratory | 69 |
| Sylvania Electric Products, Inc. | 70 |
| Technical Information Branch, ORE | 71-85 |
| UCLA Medical Research Laboratory (Warren) | 86 |
| University of California Radiation Laboratory | 87-91 |
| University of Washington | 92 |
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| Westinghouse Electric Corporation | 97 |

DISTRIBUTION OF REPORT UR-87 (cont.)

| Internal Distribution (Rochester) | Copy No. |
|---|---|
| Technical Report Control | 98-99 |
| Library | 100-101 |
| Dr. A. H. Dowdy | 102 |
| Dr. H. A. Blair | 103 |
| Dr. N. F. Bale | 104 |
| Dr. J. B. Hursh | 105 |
| Dr. L. L. Miller | 106 |
| Mr. G. W. Casarett | 107 |
| Dr. M. Salomon | 108 |
| Mr. F. W. Bishop | 109 |
| Dr. J. N. Stannard | 110 |
| Mr. G. A. Boyd | 111 |
| Dr. M. P. Watts | 112 |
| Dr. L. T. Steadman | 113 |
| Dr. H. C. Hodge | 114 |
| Dr. H. E. Stokinger | 115 |
| Dr. F. A. Smith | 116 |
| Dr. W. F. Neuman | 117 |
| Dr. E. Maynard | 118 |
| Dr. J. K. Scott | 119 |
| Dr. A. Rothstein | 120 |
| Dr. J. W. Howland | 121 |
| Mr. H. Mermagen | 122 |
| Mr. R. Hayes | 123 |
| Dr. M. L. Ingram | 124 |
| Dr. E. Otis | 125 |
| Dr. L. E. Young | 126 |
| Dr. G. B. Mider | 127 |
| Dr. K. E. Mason and Dr. J. G. Wilson | 128 |
| Dr. W. L. Bradford | 129 |
| Dr. H. D. Kingsley | 130 |
| Dr. W. B. Mason | 131 |



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TABLE OF CONTENTS

Page No.

## 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 $\gamma$ 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

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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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| 3460 | Theoretical Problems | W. Burkett Mason |

PROGRAM X.R.

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

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

Section Code: 3140

Authors: Kurt Altman and Kurt Salomon

Hemin Synthesis in Spleen Homogenates:

Background: It has been demonstrated in this laboratory (1) that the alpha-carbon atom of glycine is incorporated in the hemin and globin moieties of the hemoglobin molecule when glycine labeled with $C^{14}$ in the methylene carbon atom is fed to rats. Several instances of in vitro hemin synthesis are known. It has thus been shown that hemin synthesis from methylene-carbon labeled glycine takes place in rabbit bone marrow homogenates (2). It has also been shown that nucleated avian erythrocytes are capable of in vitro hemin synthesis when glycine labeled with $N^{15}$ is added to the incubation mixture (9). Since there exists histological evidence of extramedullary hematopoietic activity in the spleen (4, 7), it was thought of interest to test with biochemical methods the possibility of hemin synthesis from labeled glycine in spleen homogenates in the manner previously applied to bone marrow homogenates (2).

Method: Rabbit spleens were chosen as the source of the homogenates, spleens from several rabbits having been pooled for each experiment. A spleen homogenate from 3 rabbit spleens was prepared as follows: The spleens were homogenized in the micro-cup of the Waring blender with 25 ml of 0.9% sodium chloride solution. To the resulting homogenate 0.15 millimoles of glycine

(containing concentrations of $C^{14}H_2NH_2COOH$*, indicated in Table 1 (Page 15), 0.06 millimoles of sodium acetate and 1.5 ml of M/2 phosphate buffer, pH 7.3 were added. The homogenate was then incubated at 38°C for appropriate periods of time. After addition of 10 mg. crystalline hemin as carrier, either hemin or protoporphyrin IX dimethylester was isolated, the former according to Nencki and Zaleski (8) and the latter according to Grinstein (6). The protoporphyrin dimethylester was recrystallized three times from chloroform and once from pyridine. Hemin was recrystallized as described by Fischer (5). The determination of $C^{14}$ activity was carried out with the ionization chamber apparatus of Bale and Masters as previously described (1).

Results: The results obtained are shown in Table 1 (Page 15).

One experiment (3 hrs. incubation) was carried out in a large Warburg vessel permitting the collection of evolved $CO_2$ in NaOH with subsequent isolation as $BaCO_3$. The $C^{14}$ activity of the $BaCO_3$ thus obtained was quite low ($4.8 \times 10^2$ dis./min./millimole), indicating that only very small amounts of the methylene carbon atom of glycine were converted to $CO_2$ in spleen homogenates. The $C_{16}$-$C_{18}$ fatty acids isolated in several cases also contained significant $C^{14}$ activities although the dilution of the radioactivity was somewhat higher than that previously reported for bone marrow homogenates (3). Active protein and nucleic acid synthesis also appeared to take place in these spleen homogenates.

Summary and Discussion: The results reported here indicate that hemin synthesis can be carried out by rabbit spleen homogenates utilizing methylene-carbon atom of glycine as a precursor. Experiments are now in progress to assess

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* The authors are indebted to Dr. B. M. Tolbert of the Radiation Laboratories, University of California for making available the labeled glycine used in these experiments.

TABLE 1

| Time of Incubation Hrs. | $C^{14}$ activity of glycine added $10^6$ dis/min/millimole | $C^{14}$ activity of protoporphyrin IX dimethylester $10^4$ dis/min/millimole | Co/C** |
|---|---|---|---|
| 3 | 4.6 | 16.4 | 28.0 |
| 14 1/2 | 7.2 | 48.8 | 14.8 |
| 25 | 5.3 | 60.0* | 8.8 |

* Isolated as hemin

** Co/C = ratio of $C^{14}$ activity (dis/min/millimole) of compound added (Co) to $C^{14}$ activity of compound isolated (C), i.e., the dilution constant.

the biological significance of these findings, especially with respect to irradiation damage.

## Bibliography


1. Altman, K. I., Casarett, G. W., Masters, R. C., Noonan, T. R. and Salomon, K., J. Biol. Chem., 1948, 176, 319.

2. Altman, K. I., Salomon, K. and Noonan, T. R., J. Biol. Chem., 1949, 177, 489.

3. Altman, K. I., J. Biol. Chem., 1949, 177, 985.

4. Doan, C. A., "Bone Marrow" in Downey, H. Handbook of Hematology, III pp. 1935-9.

5. Fischer, H. and Orth, H., Die Chemie des Pyrrols, II. ist part. p. 374.

6. Grinstein, M., J. Biol. Chem., 1947, 167, 515.

7. Klemperer, P., "The Spleen" in Downey, H., Handbook of Hematology, III pp. 1646-9.

8. Nencki, M. and Zaleski, J., Z. Physiol. Chem., 1900, 30, 384.

9. Shemin, D., London, I. M. and Rittenberg, D., J. Biol. Chem., 1948, 173, 799.

PROGRAM U.

URANIUM

Problem Code: U.1 (Physical and Chemical Properties)

Section Code: 3220

Author: William F. Neuman

The polarography of uranium in citrate has been reinvestigated to correct for possible errors in previous studies which did not take into account the presence of reducible $UO_2OH^+$ present in solution at pH 4 - 4.5. The earlier studies were confirmed and a rough estimation was made of the dissociation constant for the uranyl citrate complex.

Spectrophotometric studies extended the polarographic work, established the combining ratio of uranyl and citrate ions as 1:1 and gave the dissociation constant:

$$K_c = \frac{(UO_2^+)\cdot(H_2Cit^-)}{(H)^{3/2}\cdot(\text{complex dimer})^{\frac{1}{2}}} = 0.046 \pm 0.009$$

pH 3.0 - 3.7

ionic strength $\sim$ 0.1

Problem Code: U.3 (Toxic Limits)

Section Code: 3210

Author: H. B. Wilson

The Effects of Exposure of Rabbits and Rats to Inhalation of Hydrated Uranium Trioxide Dust at Approximately 10 mg/m3 Concentration and 0.45 $\mu$ Mass-Median Particle Size

Toxic responses of rabbits and rats were studied following inhalation

of hydrated $UO_3^-(UO_3 \cdot 2H_2O)$ at 0.45 $\mu$ mass-median particle size and at $UO_3$ concentration of 10 mg/m$^3$. The hydrated $UO_3$ had been prepared by precipitation from $UO_2Cl_2$ with NaOH. Animals were exposed 6 hours daily, 5 days per week for 38 calendar days.

Chief responses in the rabbit were moderate proterinuria, slight elevations of blood NPN and urea N and slight diminution of the rate of growth; in the rat, growth arrest during the first week of exposure and moderate lung damage. Histopathological inspection revealed typical "uranium" lesions in kidneys of all exposed animals, but uncovered no significant abnormalities in other organs (lung, liver, pulmonary lymph nodes).

Mean concentrations of uranium in the lungs of terminally sacrificed animals were: rabbit, 83 $\mu$g per g wet tissue; rat, 60 $\mu$g per g wet tissue. These are unexpectedly high values since $UO_3$ should be soluble in body fluids.

The chief purpose of this experiment will not have been realized until these results are compared with those of a duplicate study at larger particle-size range.

Problem Code: U.4 (Fate)

Section Code: 3210

Author: C. W. LaBelle

Biological Effects of Pile Dust.

In order to provide some experimental background on which future studies of radioactive dust might be based, a sample of radioactive dust obtained from the filter system of the Oak Ridge pile was suspended in physiological saline solution and injected intraperitoneally into a group of 10 rats at a level of 40 mg dry

dust per kilogram of body weight. Two groups of control animals were prepared, one of which received an equal weight (40 mg/kg) of natural uranium dioxide, and the other left untreated.

All three groups were studied intensively for a period of a month before, and a month after the injection. Inasmuch as all the animals survived, they were allowed to remain essentially undisturbed for an additional two months, at which time one animal injected with pile dust and one animal injected with uranium dioxide had died. The entire group was then sacrificed, and the findings in the major organs tabulated.

Results:

1. Weight and Mortality. No animal died during the 30-day period arbitrarily chosen as the limit of acute response, and only two during the entire 3½-month duration of the experiment.

Growth data did not differ appreciably from that of controls (Table 1, Page 20). It will be seen that all three groups grew at the same rate before the injection and at nearly the same rate thereafter, the slight differences between the three groups being accounted for by the temporary loss in weight sustained at the actual time of injection.

2. Hematology. The hematologic data are tabulated in Table 2 (Page 20).

It will be seen that in both groups there was a slight increase in the total white count after the injection, which seems to be due to the presence of unusual numbers of disintegrating and damaged cells. The other leukocytic variables, polymorphonuclears and lymphocytes remained within normal limits of the controls. It is suggested as a possible explanation that these damaged cells represent the phagocytes which had been mobilized as a response to the presence of dust in the peritoneal cavity, and which returned to the blood stream in such

TABLE 1

GROWTH OF RATS BEFORE AND AFTER INJECTION

| | ANIMALS RECEIVING | | |
|---|---|---|---|
| | Radioactive Dust | Uranium Dioxide | No Treatment |
| | g | g | g |
| Mean growth in 30 days preceding injection | ∕ 109 | ∕ 100 | ∕ 100 |
| Mean growth in 30 days following injection | ∕ 53 | ∕ 56 | ∕ 62 |
| Immediate weight change when injected | - 11 | - 7 | 0 |

TABLE 2

LEUKOCYTIC CHANGES IN RATS AFTER INJECTION

Numbers of cells x $10^3$ gained or lost per cubic millimeter after injection

| Interval | Group | Total White Cells | Polymorphonuclears | Lymphocytes | Damaged Cells |
|---|---|---|---|---|---|
| 1 week | radioactive | ∕ 3.5 | - 0.1 | ∕ 0.4 | ∕ 3.8 |
| | uranium | ∕ 1.5 | - 0.6 | - 0.4 | ∕ 2.5 |
| 2 weeks | " | ∕ 1.6 | - 0.6 | - 1.0 | ∕ 0.9 |
| | | ∕ 1.0 | ± 0.0 | ∕ 0.1 | ∕ 1.0 |
| 3 weeks | " | ∕ 0.5 | ∕ 0.4 | ∕ 0.1 | ∕ 0.9 |
| | | ∕ 0.3 | ∕ 0.3 | ± 0.0 | ∕ 0.9 |

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[redacted]

numbers as to saturate temporarily the normal mechanisms by which such cells are eliminated.

3. Findings at Autopsy. The findings at autopsy, 3 months after the injection, are shown in Table 3 (Page 22).

The uranium-treated animals differed from the untreated controls only with respect to the kidney, which showed a moderate degree of damage. The animals treated with radioactive dust, on the other hand, showed some evidence of damage in each of the organs studied except the spleen. This is made more obvious in Table 4 (Page 23), which is constructed on the following basis: values which fall within two standard deviations of the control mean (mean $\pm$ 2 $\sigma$) are accepted as normal. Values differing from the control mean by more than 2 $\sigma$ but less than 3 $\sigma$ are classified as doubtful, while values differing from the control mean by more than 3 $\sigma$ are classified as abnormal.

Different organs manifested changes in different ways; thus the liver and lung showed physical changes and changes in relative weight, the kidney, signs of damage such as necrosis with no change in the weight of the organs, whereas the heart showed no obvious effect other than an increase in size. The criteria for abnormality are sufficiently rigorous, however, (3 $\sigma$ corresponding to a random probability of 1:1000) that the incidence of pathologic change seems well established.

The character of the damage in each organ may be summarized:

Lung - The damage visible in the lung was rather diffuse and seemed to be only an extension of the type seen to a lesser extent in the controls, viz., lesions associated chiefly with current or past infection processes. If this is true, it suggests that the increased damage is to be ascribed to a lowered resistance to normal invaders.

[redacted]

TABLE 3

APPARENT GROSS DAMAGE AND ORGAN WEIGHTS IN RATS

| Organ | ANIMALS RECEIVING | | | |
|---|---|---|---|---|
| | Radioactive Dust (N = 9) | Uranium Dioxide (N = 5) | No Treatment Mean ± Std.D. (N = 10) | |
| Lung; gross damage[1] | 2.1 | 1.0 | 1.4 | 0.7 |
| weight[2] | 0.64 | 0.60 | 0.54 | 0.06 |
| Liver; " | 2.6 | 0.0 | 0.3 | 0.6 |
| " | 3.8 | 4.0 | 4.7 | 0.4 |
| Kidney; " | 3.0 | 1.8 | 0.1 | 0.3 |
| " | 0.47 | 0.41 | 0.45 | 0.03 |
| Spleen; " | 0.3 | 0.2 | 0.1 | 0.3 |
| " | 0.40 | 0.39 | 0.41 | 0.05 |
| Heart; " | 0.2 | 0.0 | 0.0 | 0.0 |
| " | 0.37 | 0.33 | 0.31 | 0.01 |

(1) Arbitrary scale, 0-10
(2) As percentage of body weight



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TABLE 4

PATHOLOGICAL CHANGES IN RATS EXPOSED TO RADIOACTIVE DUST

| Organ | Fractions of Animals Whose Organs Were | | | Direction of Change |
|---|---|---|---|---|
| | Normal | Doubtful | Abnormal | |
| Lung | 5/9<br>5/9 | 3/9<br>2/9 | 1/9<br>2/9 | Increased weight<br>Increased "damage" |
| Liver | 2/9<br>3/9 | 4/9<br>2/9 | 3/9<br>4/9 | Decreased weight<br>Increased "damage" |
| Kidney | 5/9 | 0/9 | 4/9 | Increased "damage" |
| Spleen | 9/9 | 0/9 | 0/9 | ------ |
| Heart | 1/9 | 0/9 | 8/9 | Increased weight |

Liver - The abnormal livers were dark in color, reticulated and friable. They appeared to be swollen with blood in spite of the decrease in the total weight of the organs. Numerous black nodules were visible below the surface, usually grouped into definite zones or patches.

Kidney - The damage in the kidneys ranged from moderate hemorrhages to severe necrosis. In one animal, one kidney was completely non-functional, the other severely hemorrhaged.

Heart - No outward signs of damage were noted, but in nearly every case the organ was 10-30% larger than normal.

Conclusions: Injection of radioactive "pile dust" into the peritoneal cavity of rats produced no specific changes in the animals during the first month. After 3 months, severe degenerative changes were demonstrated in the liver, kidney and heart, and possibly the lung. These changes may be of three general types: (1) changes due to direct irradiation; (2) changes due to direct chemical action, or; (3) changes occurring as secondary effects, i.e., because of lowered bacterial resistance, accumulation of toxins, etc. It seems probable that the diversity of the response is related to the heterogenity of the dust sample. It would therefore seem advisable to attempt a preliminary fractionation of the dust sample, because of the difficulty in sorting these diverse effects during transport of radioactive dust in and through the lung.

UNCLASSIFIED

PROGRAM Be.

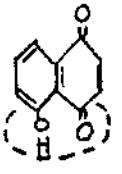
BERYLLIUM

Problem Code: Be.1 (Physical and Chemical Properties)

Section Code: 3220

Author: William F. Neuman

Having developed micromethods for Be employing alkannin and napthazarin some work has been done on elucidating the mechanism of color formation. An analogue, juglone, has been synthesized which together with other data indicates color formation is due to the existance of a chelate compound of Be and the anthraquinone. The active groups required for this compound formation appear to be:



This concept is contrary to the popular view expressed by Feigl that color formation involves adsorption of the anthraquinone on a metallic hydroxide "lake".

Considerable success has attended our efforts at purifying morin. The purified product unlike the commercial product (which contains innumerable tars and related substances) is quite specific for Be and shows a remarkable sensitivity (detection limit = 0.002 /15 ml or 1 pt per 10 billion). Thus the fluorimetric determination of Be using morin appears most promising.

Further ultrafiltration studies of Be in citrate medium have been carried out. Results will be reported subsequently.

UNCLASSIFIED

Problem Code: Be.3 (Toxic Limits)

Section Code: 3210

Author: R. H. Hall

Toxicity of Inhaled Beryllium Fluoride. Continuing the program of investigation of the toxicity of beryllium compounds by inhalation, a preliminary study has been completed in which 6 species of laboratory animals were exposed to a concentration of $10 \pm 1.4$ mg/m3 of beryllium fluoride mist. Colorimetric analyses of filter paper dust samples indicated an average concentration of 52% of that calculated gravimetrically as $BeF_2$. The toxic mist was produced by aerosolization of a solution of beryllium fluoride containing 0.52 g/ml of solute (spectrographic analysis) and having a specific gravity of about 1.2 and a pH of 1.5. The mean temperature in the chamber during the exposure period was $70.6 \pm 1.0^{\circ}$ F and the mean relative humidity, $38 \pm 3.2\%$.

A total of 102 animals was exposed, comprising 6 cats, 6 male dogs, 20 guinea pigs, 20 female mice, 10 rabbits and 40 rats. The guinea pigs were equally divided in 2 groups with respect to sex. Two groups of rats were exposed, each comprising 10 individuals of each sex, 1 group made up of young adults, the other of year-old animals. Preceding exposure to the toxic mist, the animals were conditioned for two weeks in a mist-free exposure chamber. During this time, they were subjected to the same conditions of temperature and relative humidity, daily handling and food deprivation as during the exposure period. The animals were weighed at regularly scheduled intervals and blood specimens were obtained from each of the 6 dogs for determination of serum protein, plasma fibrinogen, serum alkaline phosphatase and blood urea nitrogen concentrations and for complete cell counts and determination of hemoglobin concentration.

hematocrit and sedimentation rate. Data obtained during the conditioning period provided an index of the suitability of the particular animals for further experimental study as well as control values of the several toxicologic indices.

The animals were exposed 6 hours daily, 5 days each week. It was planned to continue the experiment for 3 months, but exposure was terminated after 63 hours because of the high percentage of mortality. Deaths occurred during exposure in all species except cats. One dog died and another was sacrificed in a moribund condition after 5 days of exposure. Cumulative mortality among the guinea pigs was 35% with no significant difference between males and females. Thirty per cent of the mice died within 60 hours of exposure. The lowest

the dogs likewise lost weight, ranging between 8.2 and 20.2% of their initial weights. Since one dog died and another was sacrificed after the 5th day of exposure, the data for this species are not comparable with those for the cats. All the mice and all the rats lost weight with the exception of the young females of the latter species. The weight loss of the young adult male rats amounted to 6.6% of their maximal pre-exposure weights. Among the one-year old rats, the males exhibited a weight loss of 22.3%, the females 18.6% of their initial weights.

All of the dogs exhibited an increase in red blood cell count, hemoglobin concentration and hematocrit, which was interpreted as evidence of hemoconcentration resulting from severe pulmonary irritation and edema. Correlated with these hematologic changes, there was an increased concentration of serum alkaline phosphatase, plasma fibrinogen and blood urea nitrogen without significant changes in the concentration and distribution of the serum protein. Because of the extremely rapid onset of the changes noted in serum alkaline phosphatase, plasma fibrinogen and blood urea nitrogen concentrations, as well as the obviously severe effects observed in the dogs, it seems probable that all of the clinical-chemical findings are referable to hemoconcentration.

All of the animals surviving after 63 exposure hours were sacrificed. Autopsies were performed and bone and tissue sections were taken for microscopic examination. In addition, autopsies were performed and tissue sections were prepared from the organs of animals that were sacrificed or that died during the course of the exposure period. No lesions attributable to toxic effects of beryllium were observed in any organ other than the lung in any species. The lung changes were inflammatory in nature and similar to those previously observed in animals exposed to $BeSO_4 \cdot 6H_2O$ mist. The lesions were similar in character in all species but their extent and severity varied considerably. The most

extensive pulmonary damage was observed in the dogs, rabbits and cats in the order mentioned. No definite pathologic changes attributable to beryllium fluoride inhalation were found in the lungs of guinea pigs and mice and only minimal effects were seen in the rat lung.

From the results of this experiment, it is clear that the fluoride of beryllium is considerably more toxic than the sulfate. The concentration, as $BeF_2$, was chosen to give about the same level of exposure to beryllium as in a previous experiment with $BeSO_4 \cdot 6H_2O$ mist at 50 mg/m3 (about 2 mg Be/m3), in which a median lethal dose for rats was attained after 2 weeks of exposure. In the present study, 50% mortality was observed among the rats in about 2 weeks after exposure to a beryllium concentration of only about 1.0 mg/m3. Beryllium fluoride is, therefore, roughly about twice as toxic as $BeSO_4$. The failure to observe histologic evidence of damage in the lungs of the guinea pigs, mice and rats is difficult to reconcile with the relatively high incidence of mortality in these species.

Problem Code: Be.3 (Toxic Limits)

Section Code: 3210

Author: H. E. Stokinger

Production of Anemia in Acute Experimental Beryllium Poisoning. This report summarizes the production of anemia in dogs and rats exposed by inhalation to beryllium sulfate and describes the nature of anemia and its trea tment with folic acid and vitamin B-12. Anemia, tentatively described as macrocytic, recently has been recorded in these laboratories as a result of the inhalation of beryllium sulfate mist, at a level of 1 mg/m3. Because this level, which was equivalent to

but 40 μg Be/m3, produced only borderline response and because the possibility existed that the anemia was an adventitious finding and a somewhat unexpected result, no particular precautions were taken to minimize factors that may have predisposed the anemia such as common parasitic infestations and inadequate nutritional status of the animals. Accordingly, the present experiment was performed in which these factors were controlled to a greater extent. A 6-week pre-exposure period was employed during which the nutritional status of the animals was brought to a maximum with dietary supplements (Lextron)*, parasitic infestations were reduced and control data on weight and hematologic and biochemical variables were followed. In addition, in order to insure a more definite response, the level of the present study was increased to approximately 4 mg $BeSO_4 \cdot 6H_2O$/m3.

Materials and Methods. Eight dogs and 40 rats were each divided into equal groups. One group of each species was placed on the usual diet; the diets of other groups were supplemented daily with folic acid as part of the study to determine the effect of this vitamin on the anemia. Dogs were administered folic acid in amounts of 10 mg per day in 2 divided doses by capsule orally. This dose later was doubled and finally two weeks prior to the end of the 60-day exposure, vitamin B-12 was administered in dosages of approximately 2.5 μg per dog per day. The rats received a dosage of folic acid amounting to approximately 2 mg per day per rat on the basis of food consumed. Weight and mortality records were kept on both species. Accurate daily food intake records were kept on the rats during exposure but only daily approximate records on the dogs. On a weekly basis, blood counts were made in quadruplicate on the dogs. Biochemical

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* A liver-stomach concentrate containing ferrous iron and vitamin B complex.

determinations on the blood of these animals included estimation of total protein, albumin and globulin, as well as alpha, beta and gamma globulins and calculation of the A/G ratio. Weekly determinations were also made of serum alkaline phosphatase and serum-dilution turbidity.

Exposure Conditions. The animals were exposed daily in an approximately 7 $ft^3$ chamber to a mist of beryllium sulfate approximating the composition $BeSO_4 \cdot 6H_2O$. The over-all concentration of mist to which the animals were exposed was 3.6 mg/m3 calculated from the spectrographically adjusted daily averages of 254 samples taken hourly throughout the 5-day week of the 60-day exposure. The chamber temperature during exposure averaged 74 F; the relative humidity averaged 46%.

Results.

Production of Anemia. Both exposed groups of 4 dogs each developed a macrocytic anemia irrespective of treatment with folic acid; accordingly, no distinction will hereafter be made between the responses of the 2 groups. The development of the condition was gradual and progressive starting in from 1-3 weeks after exposure to beryllium had begun. Anemia was demonstrated by an over-all fall in red blood count of approximately 2 million cells ($7.1 - 5.2 \times 10^6$) after 6 weeks of exposure; hemoglobin values fell from an average of 15 to 13 g% during this interval. At the same time, the mean corpuscular volume (MCV) rose on the average from 69 to 86 $\mu^3$ based on hematocrit readings. MCV showed peaks at times during exposure attaining values as high as 96 u3. Thus on these bases, anemia of a macrocytic type has been established.

Weight Response. Characteristic of acute beryllium poisoning, 7 of 8 dogs lost weight progressively. This was first noticed 10 days after the start of exposure. Losses amounted to from 0.5 to 2.7 kg in dogs averaging 9.1 kg

(range 6.8-10.8 kg). Typical of beryllium poisoning, weight loss was not related to food intake. Dogs regularly consuming their diet, frequently lost as much weight as those that consumed only part of their diet. One dog of the 8 showed no weight change during the 60 days of exposure. Toward the end of exposure, a tendency toward weight recovery was noted, although this amounted to but 0.2 kg.

Mortality. Two of 8 dogs died as a result of beryllium exposure. One dog died following 21 days of exposure, the other following 43 days of exposure. Although both these dogs had received daily supplements of folic acid, it is difficult to believe that the folic acid in any way contributed to their death.

Biochemistry. Correlated with the hematologic findings was a hypoalbuminemia in all exposed dogs. Trends were progressive and gradual in all dogs (save one in which an oscillation between normal and subnormal values occurred) and amounted to from 2-3 g%. Concomittently, a progressive hyperglobulinemia occurred in 5 of the 8 dogs, a change interpreted not as compensatory in nature but resulting from the damage to globulin-producing tissues such as the reticuloendothelial system. Likewise, a progressive rise occurred in the gamma globulin fraction in 7 of the 8 dogs. No appreciable changes occurred, however, in the total serum protein.

A progressive upward trend was noted in the variable designated as serum dilution turbidity* in 7 of 8 dogs. This finding is considered to be in agreement with the observed relative hyperglobulinemia because the serum fraction responsible for a positive test of this factor is thought to be closely related to the presence of gamma globulin in the serum in relation to the formation of abnormal lipid protein complexes.

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* Dreyfuss, F., J. Lab. Clin. Med., 33, 672 (1948)

Determination of serum alkaline phosphatase was uninformative.

In conformity with the hematologic findings no differences in response were noted in the biochemical findings between the dogs treated with folic acid and those not so treated.

Anemia in Rats. In contrast to the dogs in which weekly counts were made, only terminal blood counts were made in rats. Values were obtained on five rats exposed for a period of four weeks and on the same number exposed for a period of six weeks. Red blood counts at these periods were respectively 6 and 5.5 million compared with values approximating 8 million prior to exposure. No change, however, occurred in the hemoglobin so that the mean corpuscular hemoglobin rose from a normal of approximately 20 to nearly 30 on the average. Thus anemia of a different character has been established in a second species exposed daily to beryllium sulfate mist. It is tentatively classed as a macrocytic, hyperchromic anemia in the absence of hematocrit data.

Nature of the Anemia. A number of interesting aspects of anemia produced by beryllium have been brought to light from studies on: (1) time required for development; (2) duration; (3) changes in composition of red cell stroma and certain lipids of whole red cells; (4) mechanism of anemia production; and (5) comparisons of the anemias developed in different species.

Time Factors. At the level of 4 mg/$m^3$ beryllium sulfate, the trend toward anemia development was first noticeable after one week in some dogs and as late as 3 weeks in others. In a previous study of 1 mg, anemia developed only after from 3-8 weeks. Thus the time of onset is dependent upon the concentration at which the inciting agent is inhaled. At the 4 mg level, no positive indication of a return to normal hematologic status was indicated during the 6 weeks of continued daily exposure.

In a separate experiment (3 dogs) especially designed to determine the time for recovery from anemia after exposure to beryllium, recovery became apparent only after from 8-10 weeks and then recovery was only partial. The dogs were maintained on their usual diet of Purina Dog Chow supplemented with Evr Redy bone meal. This study is still in progress. The grade of exposure in this experiment was comparable in every way to that of the major study. Thus it is shown that the anemia produced by beryllium is probably not permanent but once well-established, recovery is slow. Whether recovery is complete cannot be stated at the present time.

Changes in Constituents of Red Blood Cell. Changes in the amounts of certain components of stroma and of whole red blood cells were noted in the above dogs, in which recovery studies were made and also in dogs exposed at a 1 mg level of beryllium sulfate. At the present time, these changes are felt to be associated with the toxic anemia. Two types of biochemical examinations have been made chiefly on dogs and in some cases on rabbits. One was the determination of protein and lipid of the red cell stroma, the other, the phospholipid and free cholesterol of the total red cell. Examination of the blood of one dog that had become anemic from beryllium exposure showed at a hematocrit of 40 volumes per cent an abnormal stroma protein to lipid ratio of 3.2. The normal protein to lipid ratio is 1.5. Stroma protein in the anemic dog was 8.4 and stroma lipid, 2.78 mg/ml; normal values are respectively 6 and 4 mg/ml.

The quantitative analysis of the total red blood cell has shown phospholipid and cholesterol to increase upon continued exposure to beryllium, free cholesterol being slower and less marked than phospholipid in exhibiting the increase. Phospholipid attained values more than 2 sigma from the normal, cholesterol slightly more than 1 sigma.

Mechanism of Anemia Production. Utilizing a staining technique developed by Nizet*, a determination was made to learn whether beryllium interfered with the maturation of the red cell. This technique involved a staining of freshly drawn heparinized blood with Brilliant Cresyl Blue and observing the cells both before and after incubation at 37° for ripening, by darkfield illumination. The immature red cells by this technique appear as yellow granular orbs, whereas the mature red cells appear as a background of dark red-brown cells.

Upon application of this technique to 3 dogs exposed to beryllium sulfate mist for 6 weeks, when anemia amounting to a decrease of 2 million cells had been established, the number of immature cells in the circulating blood was so reduced as to make a measurement of their ripening time useless. One dog showed no reticulocytes whatsoever; another showed 7 per thousand and a third, 16 per thousand. The normal reticulocyte count of dogs ranges from 30-60 or more per thousand. Thus the cause of the anemia in dogs exposed to beryllium might appear to be due to beryllium slowing the liberation of reticulocytes into the blood. Normoblastic activity has been noted, however, in bone marrow sections of dogs with beryllium anemia. Therefore, trouble may arise in an earlier stage in red cell formation such as protein synthesis by the liver. This conforms to the finding of hypoalbuminemia. No evidence has been obtained that the anemia was due to a hemolytic action or to nutritional effects.

Anemia Treatment by Folic Acid.

Rats. Results of a single experiment in which 20 rats were fed a fox chow diet supplemented with small amounts (2 mg per day per rat) of folic acid showed that folic acid prevents or retards the development of anemia in rats.

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* Acta Med. Scand., 117, 199 (1944)

Blood counts, hemoglobin concentration and calculation of mean corpuscular hemoglobin (MCH) were made on 5 rats on the folic acid diet and a similar number on the regular diet after 4 and 6 weeks of exposure to beryllium sulfate at 4 $mg/m^3$. Red blood cell counts of the folic-acid treated rats at the 4th and 6th week periods averaged 7,9 and 7.3 million, respectively; rats not receiving the folic acid supplement showed average red blood cell counts of 6 and 5.5 million at their respective periods. There appeared to be a compensatory increase in hemoglobin concentration, for average values of hemoglobin in all 4 groups of rats were indistinguishable from one another, and values of MCH accordingly rose to an average of 27 $\mu\mu g/mm^3$ in the rats not receiving the folic acid compared with an average of 21 in the 2 rat groups receiving the folic acid. The normal MCH value for rats is $20 \pm 2.5$.

Beneficial effects, other than prevention of anemia, attributable to folic acid administration have also been observed. These have included effects on general condition, food consumption, body weight, mortality and lung weight.

Food Consumption. Accurate daily measurements of food intake in groups of 20 rats each, one with and one without folic acid in the diet, showed that dietary intake was approximately 1.5 times better in rats given the folic acid than those not receiving this supplement. During the one month period in which the dietary intake was measured, the folic-acid treated rats always consumed more than 70% of the 20 grams administered per rat per day; rats on the regular diet, however, consumed commonly amounts as low as 30% daily of the 20 grams administered. After the first 4 weeks of exposure, however, less difference in dietary intake was noted.

Body Weight. Folic-acid treated rats at all times showed a weight increase; rats not receiving folic acid lost weight progressively during the

first 3 weeks of exposure, the loss amounting to 12% of their original pre-exposure weights. By the 6th week, however, this loss had been recovered. At this time, however, folic-acid treated rats had gained 18% of their pre-exposure weights.

Mortality. Among the rats on the regular diet, the mortality was 40% (8 of 20); among those on the folic acid diet, 15% (3 of 20). Deaths occurred between the 12th and 21st day. After that period, no deaths occurred although exposure continued on through the 6th week.

Lung Weight. At the conclusion of the 4th and 6th weeks, rats were sacrificed from each group for the purpose of investigating the manner in which folic acid acts to protect rats from beryllium poisoning. For this purpose, the ratio of lung weight to body weight was determined as a measure of the amount of lung injury. Sections were also taken for lung pathology and content of beryllium.

At the end of 4 weeks, the ratio of lung weight to body weight of the folic-acid treated rats averaged 0.88% in comparison with 1.09% in a similar group of 5 untreated rats, thus indicating greater amounts of edema in the animals not receiving the folic acid. Larger groups of animals sacrificed 2 weeks later showed less distinction between groups. Averaged values of 11 folic-acid treated and 7 normal diet rats showed values respectively of 1.05% and 1.14%. At the present time, no pathology report is available on these tissues.

Beryllium Content of Lung and Femur. The mean total beryllium content of lungs of folic-acid treated rats was less than that of rats not receiving folic acid; values of 4.8 and 5.9 μg per total lung were attained, respectively. No distinction between the total amounts of beryllium in the femurs of these animals were noted. These values were found at the end of 4 weeks of exposure. After 6 weeks of exposure, no difference between the beryllium content of total lung

was apparent, being 8.0 and 7.3, respectively; and again no differences between content of femur in the two groups were noted.

Dogs. In marked contrast to rats, 4 dogs, exposed daily to beryllium mist and receiving daily supplements of folic acid, showed no difference in any hematologic variable measured throughout a 6-week treatment and exposure period. Anemia developed as rapidly and to the same extent as it did in a similar number of control dogs receiving no folic acid supplement and exposed under identical conditions. Neither group varied significantly from the over-all average of the red count of 5.2 million lowered from an original average count of 7.1. Likewise no distinction was noted in the decrease in hemoglobin concentration to the 2 groups which amounted to 2 g%. Associated with these changes was a rise in the MCV in both groups of animals to approximately 90 $\mu^3$. Thus the anemia developed in dogs upon exposure to beryllium differs in 2 respects from that in rats: (1) there is a decrease in the hemoglobin concentration as well as in the red blood count; (2) these decreases were unaffected by prophylactic administration of folic acid.

Anemia Treatment by Vitamin B-12. Because folic acid was ineffective in modifying the course of anemia produced by beryllium in dogs even in comparatively large doses (20 mg per day), after a period of 6 weeks, vitamin B-12 was administered to 4 dogs subcutaneously in daily doses of 2.5 µg per day. Administration of this vitamin was made irrespective of the prior treatment of animals with folic acid which was stopped after the first injection of vitamin B-12. No vitamin B-12 was given to 2 dogs serving as controls.

Within a period of 48 hours all 4 dogs receiving vitamin B-12 showed trends toward an increase of red blood cells and reduction in MCV. No alteration was caused in hemoglobin concentration, however. After approximately 2 weeks,

administration of the vitamin partially restored the red blood count. Unfortunately, 1 of the 2 control dogs appeared to show a slight rise at this time. Whether this was due to a counting error or a spontaneous remission is undecided. Accordingly, it cannot be said from these very preliminary tests whether vitamin B-12 is capable of increasing formation of red blood cells in a beryllium-produced anemia. A longer time-study is needed.

Conclusions. The chief contributions of this study to a knowledge of physiologic action of beryllium consist of the following.

(1) An anemia is produced in dogs and rats by daily inhalation of beryllium sulfate.

(2) The anemia produced by the single toxic agent, beryllium, is not identical in both species. This is based on two observations.

(a) In dogs there is a fall in red blood count and hemoglobin concentration and a rise in red cell volume with only a slight or occasional rise, but more often a fall in mean corpuscular hemoglobin concentration, thus classifying the anemia as macrocytic without the further reference to cellular hemoglobin content. In rats, there is a decrease in red blood count but no concomittant change in hemoglobin and accordingly a distinct rise in mean corpuscular hemoglobin, leading to a classification of macrocytic, hyperchromic anemia.

(b) The difference in response to folic acid is distinct in the two species. In the dog, no effect on the anemia or any other factor occurred from the use of this vitamin. In the rat, folic acid prevented or greatly slowed the development of the anemia and in addition produced in this species beneficial response in 5 other criteria of beryllium poisoning.

Thus on a hematologic basis, there is an indicated difference in the nature of the macrocytic anemia and in addition on a mechanistic basis a still greater difference in the anemias exists. The cause of this difference awaits further work.

(3) The time for the development of the anemia varies according to the concentration of the inhaled, inciting agent. Air concentration of 4 $mg/m^3$ of beryllium sulfate requires from 1-3 weeks to precipitate the anemia; 1 mg requires periods of from 3-8 weeks. The duration of the anemia in all probability depends upon the retention time of effective concentration of beryllium in the site where synthesis blockage occurs.

(4) Anemia may be produced by beryllium retarding the liberation of reticulocytes into the blood, or by injuring the liver (or kidney) as evidenced by the reduction in hemoglobin formation, production of hypoalbuminemia and the doubtful effect of vitamin B-12 in dogs. Thus the anemia may be related to the over-all disturbance in nitrogen metabolism.

(5) Associated changes were observed in the amounts of lipid and protein of the stroma and in the phospholipid and cholesterol of the blood cell.

(6) The ability of folic acid to prevent the anemia in rats but not in dogs becomes more understandable in the light of the somewhat different type of anemia produced in the two species.

Problem Code: Be.3 (Toxic Limits)

Section Code: 3220

Author: W. F. Neuman

A comparison has been made of the toxicity of IV $BeSO_4$ in young and adult

rats. Although age was an important factor, sex did not affect the toxicity. The LD50 for young (30 day) albino rats is approximately 3 mg $BeSO_4 \cdot 4\ H_2O$/kg for adults (150 day) approximately 7.2 mg/kg.

Problem Code: Be.3 (Toxic Limits)

Section Code: 3230

Author: E. A. Maynard

Weanling rats were fed beryllium carbonate ($BeCO_3 \cdot 3Be(OH)_2 \cdot 2H_2O$) at a dietary level of 5.0% for a period of one month. These rats were then returned to a stock diet containing no beryllium. It is interesting to note that these rats developed the same sort of rachitic-like lesions as reported earlier in rats fed beryllium sulfate at a dietary level of 5.0%. When followed radiographically these animals gave the same picture of apparent healing followed by a radio-lucent area in the proximal portion of the tibial shaft.

Histopathological studies of tissues from rats fed beryllium metal and other rats fed beryllium oxide (fluorescent grade) for 30 days, at dietary levels of 0.5, 2.0, and 20.0% disclosed no changes due to beryllium.

PROGRAM F.

FLUORIDE

Problem Code: F.3 (Toxic Limits)

Section Code: 3210, 3230

Author: F. A. Smith

Acute and Pilot Feeding Studies.

Observations on the effect of dietary sodium fluoride in uranium-poisoned rats have been reported earlier (1) as have also the blood and urine fluoride levels of rats fed different levels of sodium fluoride in the diet (loc.cit). In Table 1 below are listed the fluoride contents of the diets as found by analysis of single samples taken at each level. It will be noted that the diet intended to contain 0.2% sodium fluoride, actually contained close to 0.3%.

TABLE 1

FLUORIDE CONTENT OF RAT DIETS

| Sample No. | Sodium Fluoride Theoretical | Sodium Fluoride Found |
|---|---|---|
| | % | % |
| 1 | Control | 0.01 |
| 2 | " | 0.005 |
| 3 | " | 0.005 |
| 4 | " | 0.005 |
| 5 | 0.10 | 0.09 |
| 6 | 0.20 | 0.28 |

At the conclusion of these experiments, the surviving animals were sacrificed and bone and tooth samples removed for fluoride analysis in order to determine the extent of fluoride storage at the respective levels of dietary intake. The results of these analyses are listed in Table 2 (Page 44) and the data for those rats 83 days on diet are shown graphically in Figure 1 (Page 45). In this graph, the upper dietary level has been taken as 0.3% sodium fluoride as approximated by analysis. Because only one animal survived on the 0.4% level, the fluoride contents found for these samples are not included here. These data show a progressive increase in the fluoride deposited as the level of dietary intake is increased. In general, slightly more fluoride is stored by the female, though the curves for the same tissues of the two sexes are closely similar in shape. The root and crown of the teeth deposited the greatest amounts of fluoride, followed by the epiphysis, alveolar diaphysis in descending order of amounts stored. This order of storage is a reflection of the different rates of growth of the various tissues, those showing the greater growth rate being the tissues storing the greater amounts of fluoride. A two-fold increase in stored fluoride was noted for the root, crown and alveolar bone of the male rats as the level of dietary intake was increased three-fold. The females showed a two-fold increase in the root and alveolar bone only. The epiphysis and diaphysis of both sexes and the crown of the female showed a 1.7-fold increase. The closely similar fluoride contents found for the tooth root and crown of rats on the 0.2% level for 244 and 83 days is considered to be due partly to normal attrition of the teeth and partly to the fact that the incisors of those rats on diet for 244 days were clipped. The clippings were analyzed and found to contain 1130 ppm fluoride (ash weight). The fluoride content found for the epiphysis of rats on 0.2 and 0.4 per cent

TABLE 2

FLUORIDE CONTENT OF OSSEOUS TISSUES FROM RATS FED DIFFERENT LEVELS OF SODIUM FLUORIDE

| Experimental Group | Days on Diet | No. Rats (Pooled) | FLUORIDE CONTENT IN PARTS PER MILLION, ASH WEIGHT | | | | |
|---|---|---|---|---|---|---|---|
| | | | Root | Crown | Epiphysis | Diaphysis | Alveolar |
| **WEANLING RATS** | | | | | | | |
| Controls; were 0.02% NaF[a] | 181 | 6 M | 230 | 210 | 1310 | 1120 | 1370 |
| | | 6 F | 480 | 350 | 1390 | 1270 | 1140 |
| 0.02% NaF; were controls [a] | 181 | 6 M | 1500 | 1350 | 4760 | 3170 | 3090 |
| | | 6 F | 2120 | 1840 | 4940 | 3400 | 3640 |
| New controls [b] | 271 | 5 M | 470 | 370 | 1220 | 730 | 940 |
| | | 2 F | 370 | 330 | 920 | 800 | 830 |
| 0.2% NaF[b] | 271 | 2 M | 12430 | 13090 | 14970 | 15840 | 19550 |
| **MATURE RATS** | | | | | | | |
| Control | 83 | 5 M | 230 | 240 | 1010 | 680 | 770 |
| | | 5 F | 290 | 260 | 1310 | 810 | 890 |
| 0.1% NaF | 83 | 5 M | 6310 | 6080 | 6540 | 3870 | 4740 |
| | | 5 F | 6530 | 7060 | 6790 | 4100 | 5060 |
| 0.2% NaF | 83 | 4 M | 12600 | 12950 | 11010 | 7270 | 9470 |
| | | 4 F | 13580 | 12430 | 12000 | 6850 | 10390 |
| 0.4% NaF | 83 | 1 F | 16390 | 18780 | 11690 | 9630 | 13980 |

(a) Diets were inadvertently reversed after 24 days
(b) After 244 days on fluoride diet, these rats were returned to stock diet, injected subcutaneously with 5.27 mg/kg uranyl nitrate and observed for mortality for 27 days. Survivors were sacrificed and bone removed for fluoride analysis.

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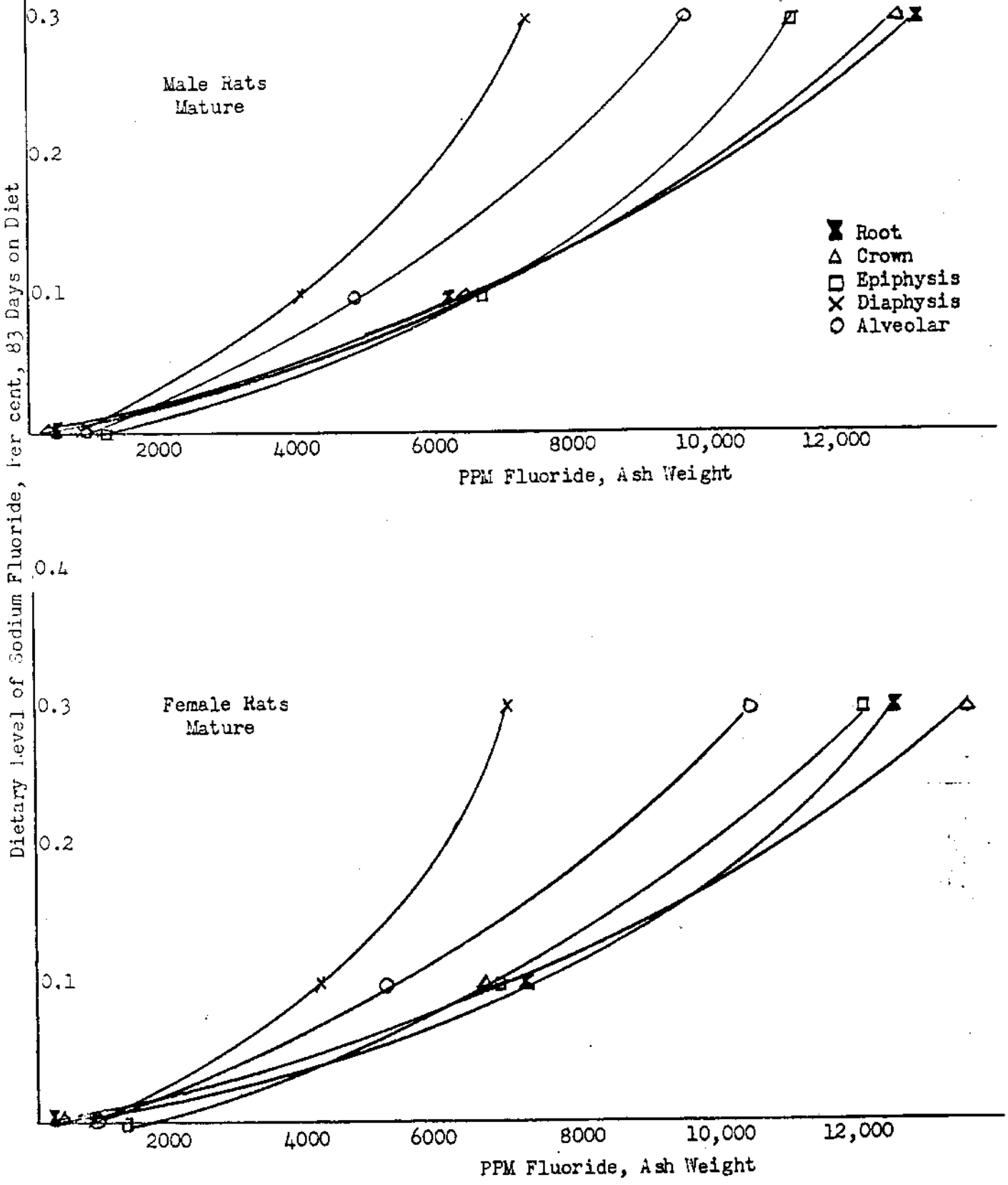
FIGURE 1. Fluoride Content of Osseous Tissues of Rats Fed Different Levels of Sodium Fluoride



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Chronic diets for 93 days suggests that a maximum storage level has been reached for this tissue. It will be noted, however, that only one sample was available from rats on the 0.4 per cent level.

(1) Quarterly Technical Report, July 1, 1948 through September 30, 1948 (UR-45)

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PROGRAM S.M.

SPECIAL MATERIALS

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

Section Code: 3210

Authors: Sidney Laskin, Paul Frank, Donald Meier

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

The existence of toxic atmospheric impurities, such as beryllium at levels of concentration well below the order of magnitude listed for the common toxic agents, has placed the sampling problem in a position of being close to its limitations of measurement and efficiency. Previously, acceptable comparative values of 90% efficiency or better are no longer significant, since size characteristics and concentrations of toxic agents may, in many cases, be similar to that of material previously passing the sampling device. In order to aid in this problem, a program has been in progress to investigate the methods for the determination of the absolute efficiency of the available sampling devices. Particular emphasis is being placed on the filter paper dust sampler and a comparative examination of various papers is planned. Also projected is further work toward the development of more efficient sampling devices and a characterization of particle sizes with relation to their effect on the efficiency of concentration sampling devices.

Most of the procedures for the determination of the efficiency of sampling methods previously available were comparative in nature and although of value in the selection of better methods, gave no indication of the absolute efficiency. Trains of similar or different samplers were also used in which the efficiency was determined as that of the ratio of the concentration in the first

sampler to the sum of the series. Various reports of such procedures at Rochester with the filter paper dust sampler in trains indicated a range of 94-99% efficiency with Whatman #41 paper for various uranium compounds. On the basis that the last sampler in the series did not collect any material, the train was considered to be 100% efficient. However, objections to this procedure have been raised on the basis that material which passes the first paper possessed size characteristics which to a large extent may have enabled it to pass through the entire train. It is of some interest in this respect to point out the good agreement of these former results with those reported below for a more refined procedure.

In order to develop a method for measurement of absolute efficiency of a sampling device, two procedures are being investigated. These include the use of a liquid nitrogen cold trap and a flame photometer as elements in a sampling train. The cold trap was introduced as means of providing a method for trapping all material that escaped the sampling device on the basis that the particles would act as crystallization nuclei for the atmospheric moisture. Liquid nitrogen was used to permit high air volumes to be drawn through the trap. After several preliminary experiments a 2 x 5" vertical-oval coil constructed of 5 turns of $\frac{1}{4}$" pyrex tubing was found to be a highly efficient sampling device for an air flow of 14 liters per minute. Since it was necessary to prove that this method was close to 100% efficient the flame photometer was developed to provide an absolute method. The flame photometer does not provide a simple field method and has the major objection of requiring that the air be pushed through the sampling train rather than drawn through it as is conventionally done in sampling. It does, however, provide a means of standardizing other methods.

The flame photometer developed was constructed from the burner and gas controls of a Beckman Flame Spectrophotometer in conjunction with the photocell

and balancing circuit of a Coleman Universal Spectrophotometer. Since the diffraction grating of the Coleman instrument was found to be unsatisfactory, a Farrand Interference Filter with a peak at 594 mμ and a half band of ± 12 mμ was substituted for studies with the sodium lines. These were chosen because of the excellent response and increased sensitivity of the instrument in this region. In the efficiency studies reported, all atmospheres contained either sodium alone or as a purposeful contaminant to permit analysis. The flame photometer was calibrated against standard $NaH_2PO_4$ solutions using the Beckman aspirator. The reliability of the instrument was determined against $NaH_2PO_4$ solution and NaCl standards. For nine repeated determinations the mean concentration was 1.37 mg $NaH_2PO_4$/cc with a standard deviation of 0.03.

The source of suspended material used in the studies was an aerosol feed with a constant leveling device as described in previous reports. The aerosol was conducted to a mixing chamber and then to the filter paper dust sampler followed by the cold trap and the flame photometer. The concentration retained on the paper was determined by weight change on a dry basis or by analysis. The amount retained in the liquid nitrogen trap was determined by analysis. The concentrations entering the flame were estimated from flame intensities by comparing them with those obtained from standard solutions of sodium chloride aspirated directly into the flame.

Only preliminary results are available for atmospheric suspensions of NaCl and $UO_2(NO_3)_2$ mixed with NaCl. Particle-size distributions were similar with size-mass medians ranging from 0.70 - 0.75 micra and with 95% of the material below 1.2 - 1.3 micra. Sampling rates varied depending upon the type of train and conditions of the experiment. Whatman #41 filter paper was used for all sampling tests.

With a mixed $UO_2(NO_3)_2$-NaCl atmosphere having an average concentration of 59.1 mg U/$m^3$ and 4.7 mg Na/$m^3$, sampled at a rate of 8.8 liters/min, the average absolute efficiency was found to be 95.6% with a range in values from 94.6 - 96.3%. A second filter paper placed in the train showed an average concentration of 3.7% with a range in values of 2.9 - 5.0%. Average concentrations of 0.5% with values from 0.1 - 1.4 g were obtained in the cold trap and 0.2% with values from 0.2 to 0.4% in the flame photometer. A source of error in this experiment was discovered when it was realized that part of the material collected in the cold trap was being blown off as the air in the trap expanded on being heated to room temperature. By allowing the air from the trap to expand through bubblers, an error up to 0.5% in the determination of the filter paper efficiency was attributable to the blow off. This error is within the range of the values obtained for absolute efficiencies. The average results, therefore, are reported for comparison with the previously reported efficiency values of Whatman #41 paper in a two-filter-paper train (95.6% compared with 95-97%). In later studies a more satisfactory method for eliminating the cold-trap error was to allow the air to expand into rubber balloons containing water so that thorough scrubbing would occur before release to the atmosphere.

With a sodium chloride atmosphere at average concentration of 29 mg Na/$m^3$ sampled at 11 liters/min., the absolute efficiency of Whatman #41 paper was found to be 96.1% with a range in values from 95.2 - 97.3%. Only one filter paper was used in this train followed by the cold trap and flame photometer. The average percentage retained by the cold trap was 3.8% with a range from 2.6 to 4.7%. Only 0.1% of the total sample was found to enter the flame photometer. These results indicate the high efficiency of the cold trap and suggest its use alone as a means of determining filter paper efficiency by conventional methods.

In another series of experiments, the flame photometer alone was used to determine the efficiency of Whatman #41 filter paper against a mixed atmosphere of $UO_2(NO_3)_2$-NaCl aerosol at an average concentration of 53.2 mg U/$m^3$ and 4.5 µg Na/$m^3$. The sampling rate was 13.9 liters/min. The average efficiency of the paper was found to be 95.4% with a range in values from 94.5 to 96.3%. Typical results are given in Table 1 below.

TABLE 1

Efficiency of Whatman #41 Filter Paper for Sampling a $UO_2(NO_3)_2$-NaCl Combined Aerosol Atmosphere at a Sampling Rate of 13.9 Liters/Min.

| Sample No. | Air Conc. mg U/$m^3$ | Air Conc. mg Na/$m^3$ | % Retained on First Filter Paper | % Passing to the Flame Photometer |
|---|---|---|---|---|
| 1 | 47.8 | 4.1 | 94.8 | 5.2 |
| 2 | 49.4 | 4.2 | 94.7 | 5.3 |
| 3 | 50.8 | 4.3 | 94.5 | 5.5 |
| 4 | 62.5 | 5.3 | 95.2 | 4.8 |
| 5 | 52.0 | 4.4 | 96.9 | 3.1 |
| 6 | 56.5 | 4.8 | 96.3 | 3.7 |

For comparative purposes a second series with a train of two filter papers and the flame photometer was studied Table 2 (Page 52). The average concentrations were 58.0 mg U/$m^3$ and 4.9 mg Na/$m^3$. The sampling rate was 10.6 liters/min. For this series, the average efficiency was found to be 95.6% with a range in values from 95.1 to 96.0%. The second filter paper retained an average value of 3.1% with a range from 2.8 - 3.4%. The average percentage entering the flame photometer was 1.3% with a range from 1.2 - 1.3%. The agreement of the efficiency value of 95.6% with that of the previous series of 95.4% was considered good despite the differences in sampling rate. Similar agreement

was found in the sum of the second filter paper and the flame photometer values of the last series indicating good performance of the flame photometer (4.4% average compared with 4.6%).

TABLE 8

Efficiency of Whatman #41 Filter Paper for Sampling a $UO_2(NO_3)_2$-NaCl Combined Aerosol Atmosphere at a Sampling Rate of 10.6 Liters/Min.

| Sample No. | Air Conc. mg $U/m^3$ | Air Conc. mg $Na/m^3$ | % Retained on First Filter Paper | % Retained on Second Filter Paper | % Passing to the Flame Photometer |
|---|---|---|---|---|---|
| 1 | 54.5 | 4.6 | 95.7 | 3.0 | 1.3 |
| 2 | 57.0 | 4.8 | 96.0 | 2.8 | 1.2 |
| 3 | 56.5 | 4.8 | 95.1 | 3.4 | 1.5 |
| 4 | 64.0 | 5.4 | 95.6 | 3.2 | 1.2 |

A preliminary finding of some interest is the fact that predrying of the atmosphere by allowing it to pass over a calcium chloride tray before sampling yielded higher absolute efficiencies than obtained in the results reported. Within the range of sampling velocities explored (8-16 liters per min.) the sampling rate at present does not appear to have a large effect on concentration determinations with the types of atmospheres studied. Variations attributable largely to differences in characteristics of the filter paper from sample to sample appear to be the greatest source of error. This factor and the effect of a wider range of sampling velocities are the subject of current investigations. Work is also in progress on modification and simplification of the testing equipment.

UNCLASSIFIED

PROGRAM I.S.

ISOTOPES

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

Section Code: 3120

Authors: L. L. Miller, W. F. Bale, C. L. Yuile, R. E. Masters, G. H. Tishkoff, and G. H. Whipple*

## Studies in Protein Metabolism in the Dog Using $C^{14}$-Labeled DL-Lysine.

### I. Synthesis and Utilization of Plasma Proteins.

Background: Studies of protein nutrition and protein metabolism are of fundamental importance in seeking an understanding of a variety of phenomena of basic concern to modern medicine. Blood protein production and utilization, wound healing, convalescence, the resistance of kidneys and liver to specific toxic agents, and the physiological burden of pregnancy are among the outstanding fields of interest in which it is already recognized that adequate protein nutrition and an understanding of protein metabolism are critically involved.

Because under normal conditions the concentrations of various concentrations of blood protein components are maintained within narrow limits by physiologic mechanisms, it was impossible by classical methods to measure either the rate of production, or the rate of disappearance of a given protein component.

The availability of synthetic DL-lysine-$\epsilon$-$C^{14}$ with $C^{14}$ in unequivocally known position (1) has enabled us to study not only the incorporation of this

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essential amino acid in the various body proteins, but also to follow the time course of the isotopically-labeled blood proteins. In addition, the apparent rate of disappearance of the labeled plasma proteins injected into normal recipient animals has been studied in an attempt to define the comparative behavior of the plasma albumin and globulin fractions. It will be seen that in these experiments the globulin fraction is turned over more rapidly than the albumin fraction on a gram for gram basis.

Method: In Experiments D-1-L, D-3-L, D-7-L, normal healthy dogs were fed the DL-Lysine -$\epsilon$-$C^{14}$ with diet protein in the form of hamburg. The appearance of radioactivity in the blood cell and plasma proteins was followed for 12 days in D-1-L and for 27 or 28 hours in D-3-L and D-7-L. In the latter two experiments $C^{14}$ excretion in the expired $CO_2$ was also followed and the $C^{14}$ distribution in the tissues was also determined.

For Experiments D-4-L and D-8-L, $C^{14}$-labeled plasma proteins were obtained as heparinized plasma from the dogs of Experiments D-3-L and D-7-L respectively. The labeled plasma was injected intravenously as a single dose, and the disappearance of the labeled proteins was followed over a period of 96 and 144 hours respectively. The plasma albumin and globulin fractions were separated and their protein content and $C^{14}$ activity determined.

In Experiment D-9-L a normal anemic hypoproteinemic dog was fed labeled lysine with diet protein and allowed to regain a normal blood protein picture. The $C^{14}$ activity distribution between the plasma albumin and globulin fractions was followed over a period of 74 days.

Results: Experiment D-1-L. - Dog 47-113. DL-lysine -$\epsilon$-$C^{14}$ 1.02 gm. (26.3) microcuries) was fed with diet protein. The dog excreted 34 per cent of the total dose of $C^{14}$ in the urine collected over a period of 24 hours with an

additional 2 per cent of the total dose in the urine of the subsequent 4 days. The feces contained only about 0.9 per cent of the total dose which indicates the completeness of absorption of the fed amino acid.

The relative quantitative distribution of the activity in the tissues of this dog sacrificed under ether anesthesia 17 days after the $C^{14}$ lysine feeding can be indicated by dividing them into three groups:

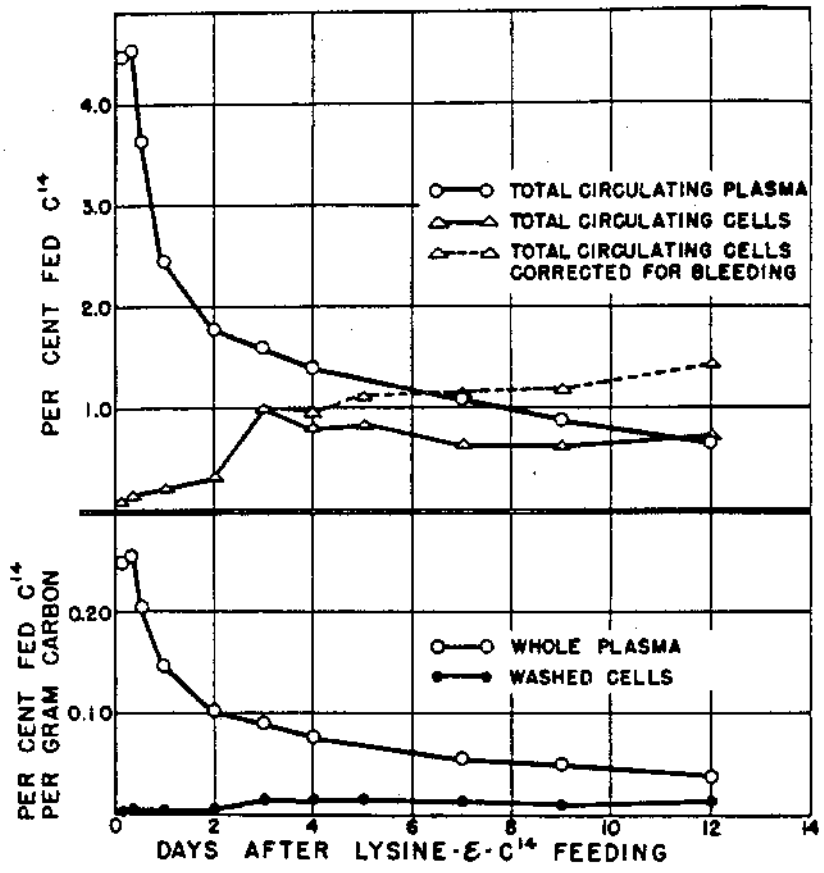
Group 1: Tissues contain 0.030 to 0.015 per cent of the administered $C^{14}$ per gm. carbon and includes liver, kidney, spleen, and pancreas in order of decreasing activity.

Group 2: Tissues contain 0.015 to 0.005 per cent of the fed $C^{14}$ per gm. carbon and includes lung, cardiac muscle, stomach, skeletal muscle, colon, duodenum, jejunum, ileum, bone marrow, adrenal, and thyroid in order of decreasing activity.

Group 3: Tissues contain less than 0.005 per cent of the fed $C^{14}$ per gm. carbon and includes vertebral bodies, ribs, aorta, lymph node, and eye in order of decreasing activity. These tissues were blood-free as the result of viviperfusion.

Figure 1 (Page 56) presents the $C^{14}$ activity of the blood cells and the plasma as estimated from samples taken over the first 12 days after the $C^{14}$ lysine feeding. It is at once apparent that the per cent fed $C^{14}$ per gm. carbon 24 hours after the labeled lysine feeding is more than twenty times as great as that of the blood cells. At this time 85 to 90 per cent of the plasma activity is found in the plasma proteins (as measured in later experiments), hence it may be said that in the non-anemic normal dog the red cell proteins acquire a considerably lower specific activity than the plasma proteins. Remembering that the blood of a normal dog contains about five times as much red cell protein as plasma protein, we may conclude that the total $C^{14}$ activity in the plasma proteins at 24 hours is about four to five times greater than that in the blood cells.

FIGURE 1




$C^{14}$ distribution in plasma and cells after lysine-ε-$C^{14}$ feeding. Experiment D-1-L. Substantial amounts of blood were withdrawn 3, 4, 5, and 9 days after the feeding of labeled lysine with hamburg.

The specific activity of the plasma protein takes about 4 days to fall to a level equal to one-half that seen at 24 hours. This is in good agreement with estimates obtained from a study of the disappearance of transfused $C^{14}$-labeled plasma. Extrapolation of the plasma activity curve to the day of sacrifice places the final plasma activity at a level slightly below that of but one organ - the liver which has long been suspected as the organ most actively involved in plasma protein production if not in plasma proteins turnover and reconversion to other protein metabolites.

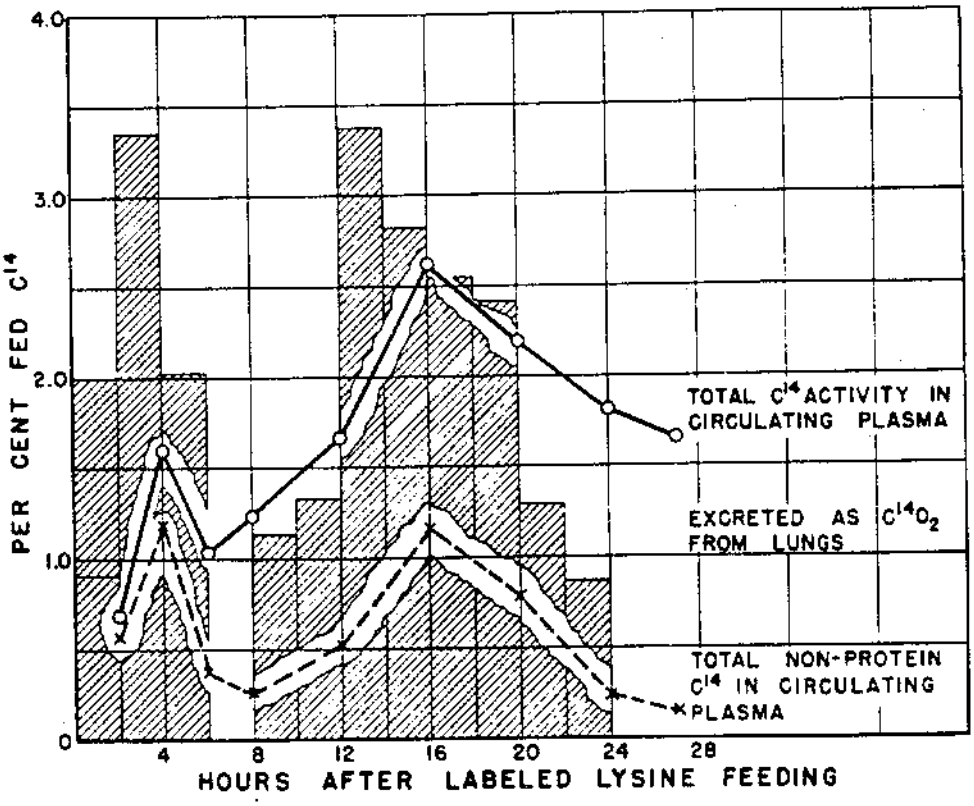
The blood cell $C^{14}$ content rises to a maximum over a period of 3 days and is there maintained at a comparatively constant level during the course of the experiment.

Experiments D-3-L and D-7-L. - Dogs 47-236 and 44-98 represent a more detailed short-term study of the varying distribution of $C^{14}$ activity in the tissues and plasma protein fractions. Experiment D-3-L differed from D-7-L only in the fact that in D-3-L the dog was given only water for 48 hours before the feeding containing the $C^{14}$-lysine while in D-7-L the dog had received its regular daily ration of kennel diet in the days prior to the experiment.

After being fed 2.00 gm. of DL-lysine-$\epsilon$-$C^{14}$ (51.2 microcuries) with 180 gm. of lean hamburg, the dog in D-3-L excreted 28 per cent of the fed dose of $C^{14}$ in the expired air and 33 per cent in the urine collected over the 24 hours of the experiment. The dog in D-7-L received a similar dose of DL-lysine-$\epsilon$-$C^{14}$ in about 150 gm. of lean hamburg and excreted 28.5 per cent and 25.1 per cent of the fed dose of $C^{14}$ in the urine and expired air respectively.

Figure 2 (Page 58) shows the $C^{14}O_2$ excretion and the non-protein $C^{14}$ activity of the plasma in D-7-L. It is clear that the radioactivity of the exhaled $C^{14}O_2$ closely parallels the level of the non-protein $C^{14}$ activity of the

FIGURE 2




Experiment D-7-L. Blood plasma $C^{14}$ content compared with $C^{14}$ in expired air (shaded columns). Drop in $C^{14}$ activities after 4 hour period related to vomiting.

plasma. The minimum in both occurs at a time closely corresponding to the point at which the dog regurgitated and was refed a large compact bolus of the food ingested at the start of the experiment. Figure 2 (Page 58) is representative of D-3-L as well as of D-7-L.

The tissue distribution in these dogs in order of decreasing activity is qualitatively and quantitatively comparable as shown in Table 1 (Page 60). Dogs killed 25 and 27 hours after feeding of DL-lysine-$\epsilon$-$C^{14}$.

The tissue distributions of these short experiments differ from the longer D-1-L Experiment in the activity of the gastro-intestinal tract tissues which are among the most active tissues of the short experiments and only of intermediate activity in the longer experiments. The liver, kidney, pancreas, and spleen are seen to maintain their positions of highest activity.

Figure 3 (Page 61) shows the specific $C^{14}$ activity of the plasma protein fractions (expressed in terms of the per cent fed $C^{14}$ activity per 100 ml. of plasma) as a function of time; in the case of both dogs more total activity is found in the globulin than in the albumin of 100 ml. of plasma. The chemical A/G ratios showed no significant changes while the $C^{14}$ A/G ratios for D-7-L showed progressive decrease. This indicates that the $C^{14}$ ratio is decreasing while the extent of $C^{14}$ incorporation in both protein fractions is still increasing. This may be a result of a higher lysine content of the globulin fraction and a reflection of a greater rate of formation in grams of the globulin fraction. The results of Experiments D-4-L, D-8-L, and D-9-L point to the latter interpretation.

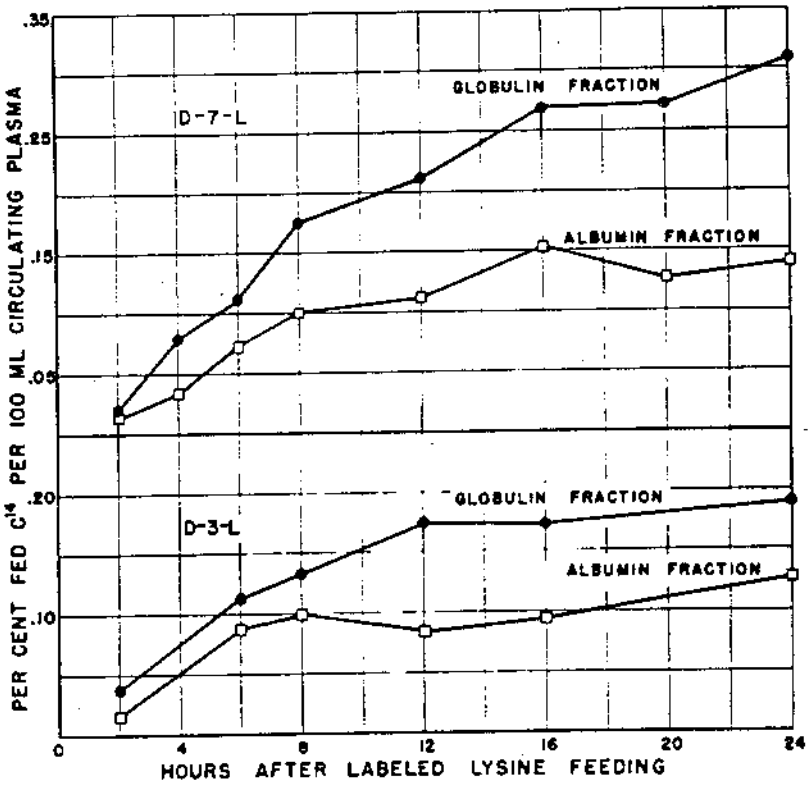
Although not shown in Figure 3 (Page 61) and Figure 4 (Page 62), the extent of incorporation of $C^{14}$ into the fibrinogen fraction at 24 hours was found to be 0.12 per cent of fed $C^{14}$ per gm. carbon.

TABLE 1

BLOOD-FREE TISSUES ARRANGED IN ORDER OF DECREASING ACTIVITY

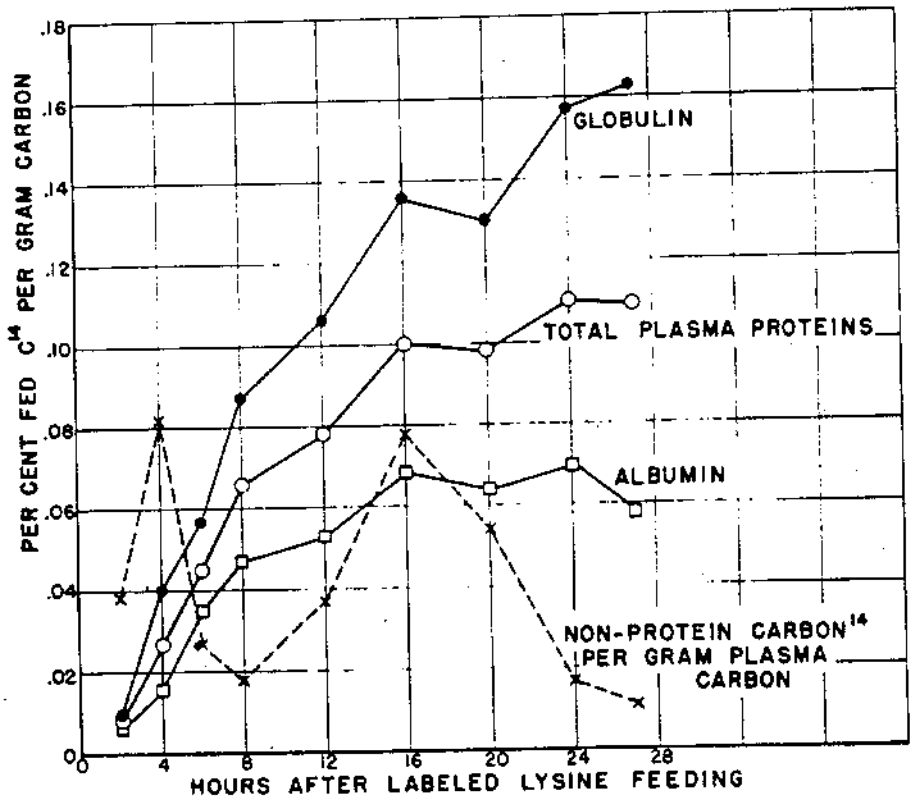
| | Tissue Activity in per cent fed $C^{14}$ activity per gm. carbon | Tissues of D-3-L | Tissue activity in per cent fed $C^{14}$ activity per gm. carbon | Tissues of D-7-L |
|---|---|---|---|---|
| Group 1 | 0.052 to 0.020 | Duodenum, Jejunum, Liver, Kidney, Gastric Mucosa, Colon Ileum, Pancreas, Parotid Gland, Esophagus, Vertebra, Spleen, Cervical Lymph Node | 0.087 to 0.020 | Duodenum, Jejunum, Ileum, Liver, Kidney, Lung, Spleen, Pancreas, Colon, Stomach, Esophagus, Cardiac Muscle |
| Group 2 | 0.020 to 0.010 | Lung, Ovaries, Thyroid, Adrenals, GI Content, Cardiac Muscle, Sciatic Nerve, Thymus, Temporal Muscle, Tongue, Femur, Eye (-Lens), Rib, Urinary Bladder | 0.020 to 0.010 | Urinary Bladder, Temporal Muscle, Diaphragm |
| Group 3 | Below 0.010 | Diaphragm, Trachea, Uterus and Bones, Parietal Bone, Thigh Muscle, Bile, Gall Bladder, Stomach Muscle, Spinal Cord, Mesentery (o) | Below 0.010 | Brain, Thigh Muscle, Spinal Cord |

FIGURE 3




Experiments D-3-L and D-7-L. Comparison of $C^{14}$ incorporation in plasma albumin and globulin fractions following feeding of lysine-$\epsilon$-$C^{14}$ with hamburg.

FIGURE 4




Experiment D-7-L. Incorporation of $C^{14}$ in plasma carbon fractions following feeding of lysine-$\epsilon$-$C^{14}$ with hamburg.

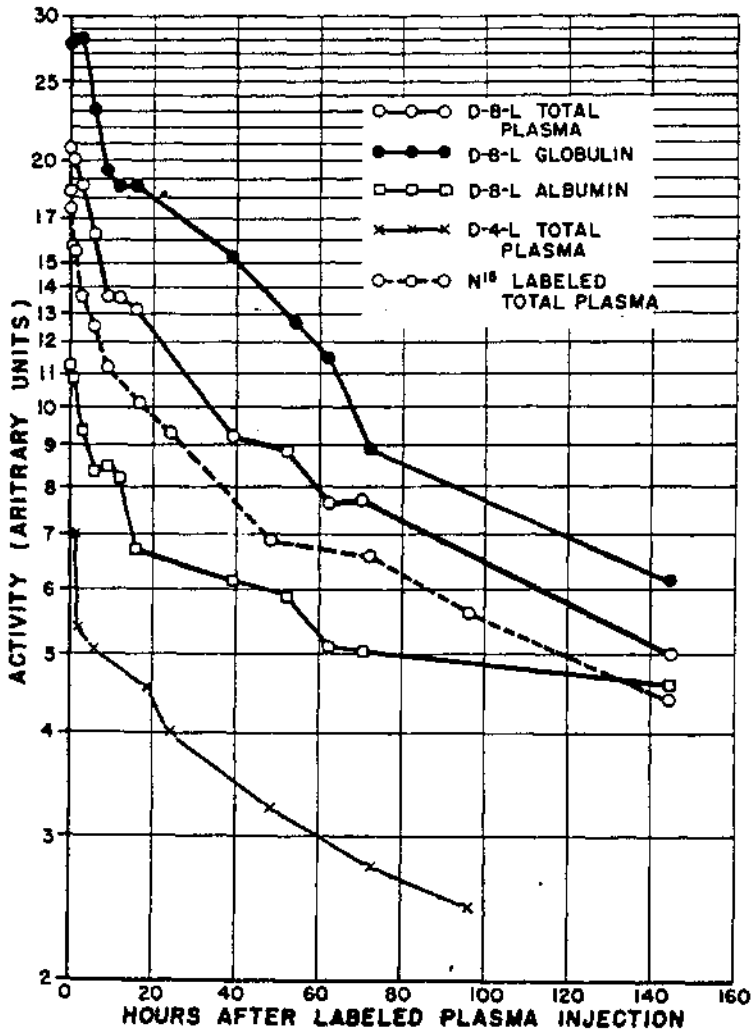
Experiments D-4-L and D-8-L. - Figure 5 (Page 64) presents the observations made on two normal dogs, 44-98 and 46-S-A after they were given single, large (133-200 ml.) intravenous injections of heparinized plasma obtained immediately prior to sacrifice from dogs in Experiments D-3-L and D-7-L. Figure 5 shows the disappearance curves of $C^{14}$ specific activity for total plasma protein in the recipient dogs. A similar disappearance curve established by the use of plasma protein labeled with $N^{15}$ (9) is included for comparison. Disappearance curves of $C^{14}$ specific activity in albumin and globulin fractions are shown for Experiment D-8-L. It is obvious that the $C^{14}$ activity of the globulin fraction decreases at a greater rate than that of the albumin fraction. The data from protein fractionation from Experiment D-4-L, not included in the figure, were qualitatively similar.

Experiment D-9-L. - Dog 44-10. This experiment presents a long-term (74 days) study of the plasma protein albumin and globulin fractions. The dog 44-10 was depleted of plasma protein and hemoglobin (2) and then fed a total of 1.576 gm. DL-lysine-$\epsilon$-$C^{14}$ (44.5 microcuries) in three equal doses on successive days.

Figure 6 (Page 65) shows the per cent of the fed $C^{14}$ per gm. carbon of the plasma proteins as a function of time. It is at once clear that not only is a larger amount of the fed $C^{14}$ incorporated into the globulin fraction, but also that the activity in the globulin fraction drops off more rapidly than the activity of the albumin fraction.

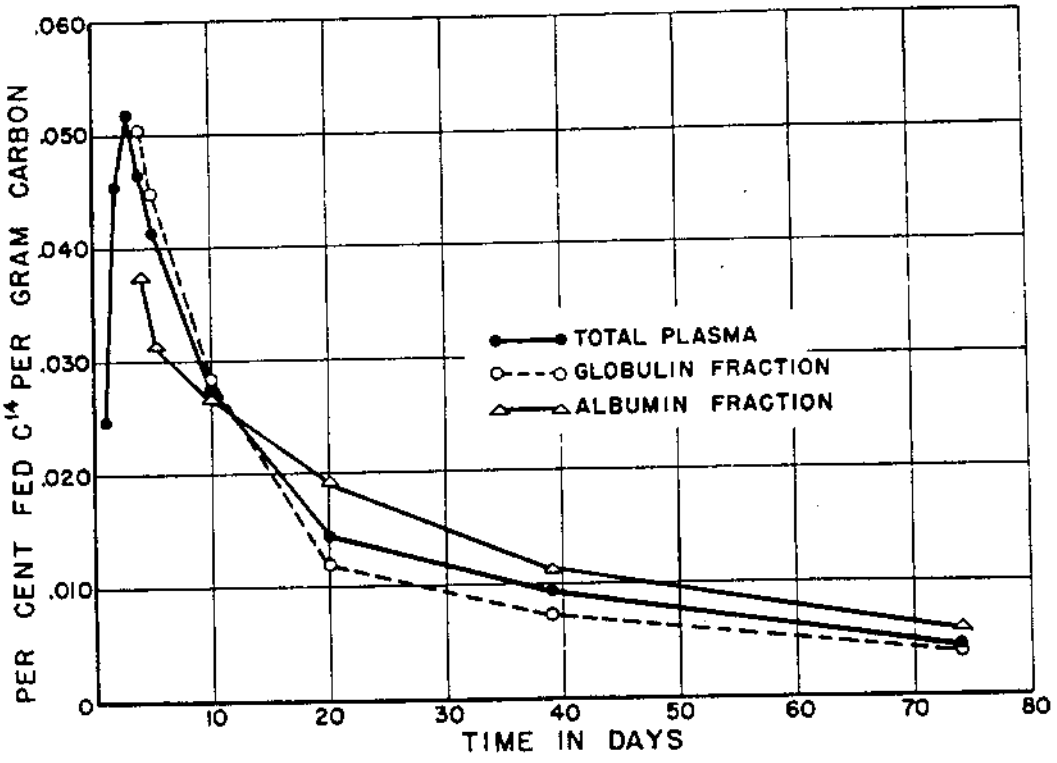
Discussion. - Little is known of the in vivo metabolism of the essential amino acid lysine. Schoenheimer and his colleagues (16), using L-lysine labeled with $N^{15}$ in the alpha amino group and deuterium along the carbon chain, found it unique among the amino acids they studied in that transamination did not occur.

FIGURE 5




Rate of decline in specific activity of plasma protein and protein fractions following transfusion of labeled plasma into normal dogs.

FIGURE 6




Experiment D-9-L. Curve showing more rapid decline in specific activity of globulin fraction of plasma than of albumin fraction following the feeding of lysine-$\epsilon$-$C^{14}$ to a dog originally anemic and hypoproteinemic.

In their experimental animals, rats, no reamination or synthesis of the carbon chain took place. Also the unnatural D-lysine was not utilized as a source of protein lysine (3). These workers did not make a detailed study of the separate organ proteins in their experimental animals. Lysine was chosen for the investigative program here because this apparent metabolic individuality makes it distinctive as a protein label. Labeled lysine isolated from a protein will be the same entity that was fed or otherwise administered to an experimental animal.

The data presented here bear on the metabolic relationship of tissue and blood proteins, the comparative rates or turnover of the plasma protein albumin and globulin fractions, and the time necessary for hemoglobin formation in the intramedullary maturation of the red cell.

Data from Experiments D-3-L and D-7-L show that the general metabolic fate of the $C^{14}$ of the lysine-$\epsilon$-$C^{14}$ was similar in these two normal dogs sacrificed 25 and 27 hours respectively after the amino acid was fed. About one-third of the fed $C^{14}$ was incorporated in blood and tissues, predominantly as protein, about one-third appeared in the expired $CO_2$, and the remainder appeared in the urine. The high urine content is undoubtedly related to the D-lysine component of the racemic mixture fed. Data from Experiment D-7-L presented in Figure 2 (Page 58) show the parallel relationship between non-protein $C^{14}$ circulating in the plasma and rate of $C^{14}O_2$ excretion in expired air. This finding was closely duplicated in D-3-L.

The pattern of $C^{14}$ distribution in tissues is basically similar to the pattern reported by other investigators following the feeding of tagged amino acids (4, 5, 6). It confirms the expectation that the finding that an organ has a high early uptake of one amino acid will in general be repeated when studies on other amino acids are carried out.

The high $C^{14}$ content noted in the tissues of the gastro-intestinal tract, particularly the duodenum, in the animals sacrificed 1 day after labeled lysine feeding is similar to findings reported after feeding other labeled amino acids including glycine (4), phenylalanine (5), and methionine (6). Some have concluded that a high concentration in the duodenum of the labeled material fed was referable to its functional activity in secreting enzyme proteins. The generally high intestinal tract activity suggests that the very rapid normal rate of regeneration of the mucosa may be of even greater significance.

Data presented by us elsewhere (7, 8) in a preliminary form indicate that this $C^{14}$ found in tissue is predominantly in protein and concentrated largely but not exclusively in the lysine residues of the protein. Significant amounts of $C^{14}$ are found in other amino acids including the carbon chains of glutamic acid, aspartic acid, and for some tissues, arginine.

Synthesis and Utilization of Plasma Protein. - The data presented here are perhaps of primary interest because of the information that can be derived concerning the rate of production and utilization of the blood proteins, particularly the protein components of the plasma.

First let us consider the plasma proteins as a whole. In the two Experiments, D-4-L and D-8-L, (Figure 5, Page 64) in which the $C^{14}$ content of the plasma proteins was followed after the transfusion of $C^{14}$-labeled plasma to recipient animals, one notes that the decline decreases with time. In the first 24 hours the rate of decline is such that if continued at the same average rate the level would have reached 50 per cent of its original value in 28 hours in Experiment D-4-L and 29 hours in Experiment D-8-L. The decline during the next 72 hours in Experiment D-4-L is such as to correspond to a rate of reduction of specific activity of 50 per cent in 100 hours. For Experiment D-8-L a rate of

reduction of 50 per cent in 120 hours can be computed from the decline in specific activity between the 39th and 144th hours.

It is to be noted that results such as these can only be produced by a preferential loss of the injected protein or by a dilution of the circulating plasma proteins by newly formed unlabeled proteins in the recipient animal. A non-specific mass movement of proteins out of the circulation would have no effect on $C^{14}$ abundance per gm. carbon, i.e., specific activity. This is pertinent since in these animals, otherwise essentially normal, 133 ml. and 200 ml. plasma respectively were injected in excess of these animals' normal plasma volume. Assuming that these animals treated the injected plasma proteins in a manner indistinguishable from their own proteins, one is led to the conclusion that they did dilute or replace the plasma proteins circulating at the time of injection by about 50 per cent new plasma proteins in 30 hours and about 75 per cent in 150 hours.

These results are not inconsistent with results previously reported following the injection of labeled plasma proteins into recipient animals. Fink and co-workers (9) have reported that in similar experiments where plasma proteins labeled by $N^{15}$ contained in lysine residue were injected into normal dogs, the $N^{15}$ concentration per gm. plasma protein had decreased to about 50 per cent of the initial value in 24 hours and about 25 per cent of the initial value in 6 days. Very similar results are reported by Fine and Seligman following the transfusion into dogs of proteins labeled with radioactive sulfur (10). Also Heidelberger and co-workers (11) found that the rate of loss of injected antibody protein in rabbits was such that of the amount circulating 20 minutes following the injection, 62 per cent remained in circulation after 48 hours and 17 per cent after 168 hours despite a large bleeding and replacement transfusion between the two

determinations.

The fact that, in the experiments reported here as well as those mentioned above, the rate of decline in specific activity is not linear when plotted on a semilog paper suggests either that different components of the plasma proteins are utilized and regenerated at substantially different rates, a question discussed in more detail later, or that the circulating plasma proteins as a whole first come into equilibrium with a labile pool of protein of about equal amount at about 24 hours after injection. The importance of the latter mechanism is suggested by the observation, shown in Figure 5 (Page 64), that the two principal fractions of the plasma proteins, albumin and globulin, each show this more rapid early fall in specific activity.

There exists a considerable amount of extravascular, extracellular protein in solution that may be collectively designated as "lymph protein". Studies on this protein (12) indicate that it is in constant interchange with plasma proteins and that if the plasma proteins were to come into equilibrium with it, the dilution would roughly account for the more rapid early phase of the decline in plasma protein specific activity. The decline in specific activity of plasma proteins produced by such a simple mixing with soluble "lymph" proteins ought not in any fundamental sense to be considered a true metabolism or "turnover" of plasma proteins. Thus these data suggest that the true time during which half the amino acid residue of the plasma proteins are replaced, presumably by the synthesis of new plasma protein molecules, is of the order of 100-120 hours. This mobilization of new plasma proteins occurred in spite of a preceding 48 hour fast in the recipient animal of Experiment D-4-L and the injection of substantial excesses of plasma in both animals, factors that should suppress rather than stimulate the mobilization of new plasma proteins.

Estimates by other authors, usually made from measurements of the abundance of labeled plasma proteins as a function of time following the feeding or injection of a tagged protein precursor, have tended to suggest that the rate at which plasma proteins are utilized and replaced in the circulation is considerably slower than is consistent with the maximum 100-120 hour period for utilization and replacement of 50 per cent of the plasma proteins reported above and derived from labeled plasma injection studies. For example, Schoenheimer, Ratner, Rittenberg, and Heidelberger (15) suggest about 2 weeks as the time necessary for such replacement in the rabbit, according to their terminology, the half-life of the plasma proteins. Data of this type obtained following the feeding of $C^{14}$-labeled lysine are found in Experiments D-1-L (Figure 1, Page 56), D-9-L (Figure 6, Page 65), and in Figure 1 (Page 76) and Figure 2 (Page 77) of the accompanying paper.

Attempts to deduce a turnover rate from such data obtained following feeding of a labeled amino acid are likely to give the erroneous impression that the turnover rate is slower than is in fact the case. This is because the labeled plasma proteins as they are metabolized and removed from circulation are replaced not by entirely unlabeled proteins, but rather by proteins again incorporating some of the labeling materials which was initially built into other tissue proteins.

This point is illustrated by Experiment D-1-L. Between the third and the ninth days some 300 ml. of plasma were removed containing approximately 53 per cent of the $C^{14}$ circulating at the beginning of this period. This total protein deficiency was made up by the animal so rapidly by the synthesis of new plasma protein that there was no appreciable drop in total plasma protein concentration. Since the plasma removed accounts for the whole of the decline

in $C^{14}$ concentration per gm. plasma protein carbon over this period, any plasma proteins leaving the blood stream for metabolic purposes must have been replaced by new plasma protein of virtually the same $C^{14}$ content.

The figure of 120 hours for utilization and replacement of one-half of the plasma proteins corresponds to an upper average time of existence of a protein entity in the plasma of 174 hours. The period during which an amount of plasma protein is synthesized corresponding to the total circulating plasma protein probably is not greater than 240 hours.

The data from Experiment D-8-L plotted in Figure 5 (Page 64) and the essentially similar results of Experiment D-4-L not presented in detail here on the specific activities of albumin and globulin fractions after injection of labeled plasma show that there is a more rapid decrease of $C^{14}$ concentration per gm. globulin than per gm. albumin. The two curves are alike in that neither is linear when plotted on semilog paper and that both show a more rapid initial decline in specific activity corresponding in magnitude to a replacement in the plasma during the first 20 hours of about 40 per cent of both circulating albumin and globulin. It was earlier suggested that this rapid initial decline represented a mixing with extracellular lymph protein. Over a period of 144 hours, however, the $C^{14}$ abundance per gm. globulin carbon had declined to 22 per cent of its initial value, that of the albumin fraction to only 40 per cent of its initial value.

Data presented in Figure 3 (Page 61) and Figure 4 (Page 62) from Experiments D-3-L and D-7-L show that in presumably normal dogs 24 hours after labeled lysine feeding, the amount of $C^{14}$ incorporated into the globulin fraction of plasma protein was greater than that incorporated into the albumin fraction - about 50 per cent greater in the first experiment, and 100 per cent greater in

the second. Also in Experiment D-9-L (Figure 6, Page 65) a dog depleted of plasma and reserve proteins showed a similar response. In this instance the amount of $C^{14}$ per gm. carbon was about 40 per cent higher in the globulin than in the albumin fraction 24 hours after the last of three daily feedings of $C^{14}$-labeled lysine. Subsequently on a stock diet the specific activity of the globulin fell more rapidly than that of the albumin so that 6 days later they were equal and in another 9 days the specific activity of the globulin was only 63 per cent of the albumin activity.

Such relative changes in specific activity, namely the greater incorporation of $C^{14}$ into the plasma globulin than albumin during the period when this material was available for synthesis and more significantly the considerably shorter period required for the disappearance of labeled globulin than albumin, indicate a more rapid metabolic turnover of the components comprising the globulin fraction than of the albumin fraction. This suggests that an animal forced to manufacture excessive amounts of plasma proteins by extensive plasmapheresis would be more able to maintain plasma globulin than plasma albumin at something approaching normal levels. That this is in fact true has been verified by studies of plasma albumin and globulin levels following plasmapheresis in dogs (13).

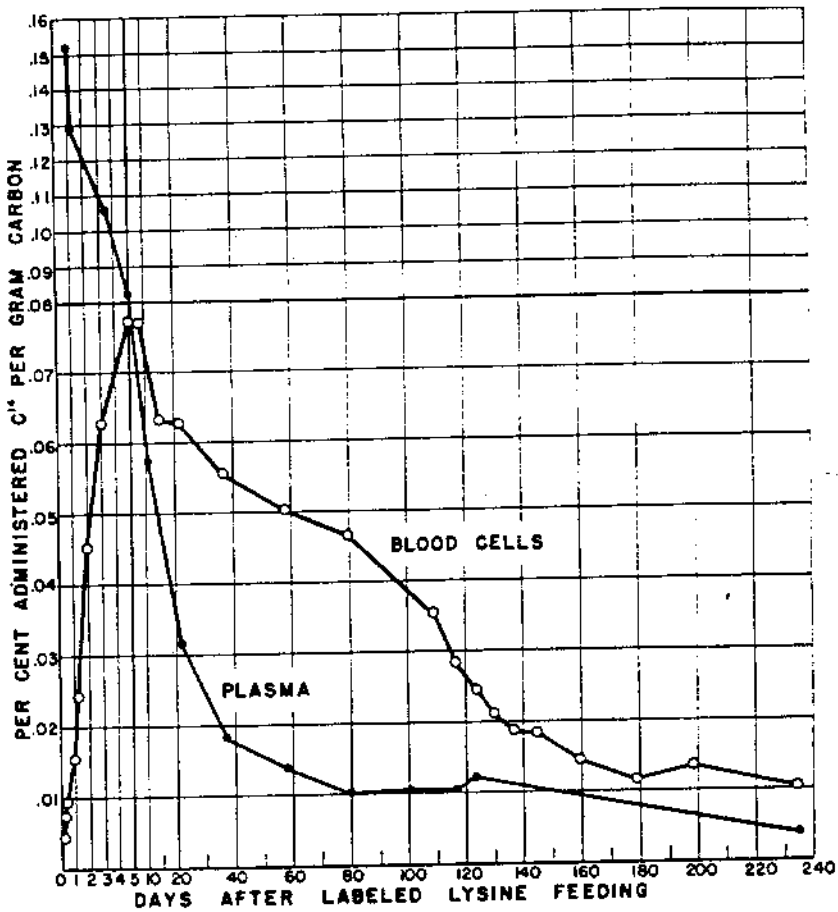
Synthesis of Blood Cell Proteins. - In Experiment D-1-L with a dog essentially normal except for a preceding 72 hour fast as shown in Figure 1 (Page 56), about 70 per cent of the total incorporation of $C^{14}$ that occurred in hemoglobin in a 12 day period took place in the first 3 days. In Experiment D-2-L described in an accompanying paper, following the feeding of $C^{14}$-labeled lysine to a dog both anemic and hypoproteinemic, of the maximum incorporation of some 6.8 per cent of the fed $C^{14}$ in blood cells over a 22 day period, some 80 per cent took place in the first 5 days.

concentration fell from 6.75 to 4.71 gm. per 100 ml. plasma. Weight fell from 15.5 to 13 kilos.

The dog was given, by mouth, mixed with 100 gm. of hamburg, 0.58 gm. of DL-lysine which contained 15 microcuries $C^{14}$ in the epsilon carbon position. The lysine synthesis had been carried out as described by Helmkamp and co-workers (7). The dog took this feeding eagerly. After 4.5 hours a feeding of 400 gm. of the basal diet low protein biscuits was supplemented with an additional 50 gm. of hamburg. The dog received daily for the next 3 days 400 gm. of biscuits plus 100 gm. hamburg. The dog was then returned to a standard kennel diet adequate for the rapid regeneration of plasma and blood cell proteins. Under this regime the red cell hematocrit rose to 48 per cent in 2 weeks and 52 per cent at the end of 5 weeks. Plasma proteins rose from 4.71 gm. to 5.9 gm. per 100 ml. plasma in 2 weeks and 6.75 gm. per 100 ml. in 5 weeks.

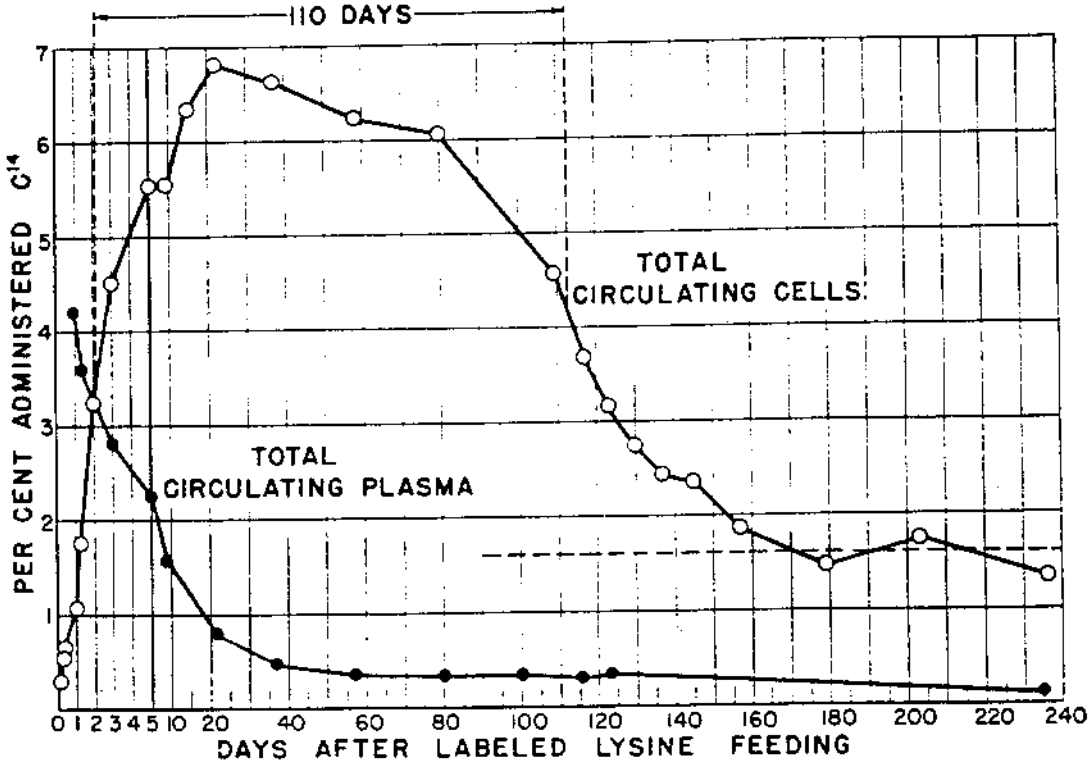
Blood samples of about 15 ml. each were withdrawn and mixed with 0.1 ml. of saturated sodium citrate at times indicated by the points on Figure 1 (Page 76) and Figure 2 (Page 77). The blood samples were centrifuged to separate plasma and the cells then washed twice with normal saline. No effort was made to separate erythrocytes from other centrifugable, formed elements of this blood. One gram portions of dehydrated blood cells and the dried solids from about 7 ml. of plasma were used for individual $C^{14}$ measurements. The carbon of such samples was converted to $CO_2$ by a wet oxidation and introduced into an ionization chamber of 1200 ml. volume. The volume of evolved $CO_2$ was determined. Ionization current measurements were made with a dynamic condenser electrometer (1). Quantitative urine collections were made with a metabolism cage and aliquot portions were analyzed for $C^{14}$. Single radioactivity determinations were made for plasma and urine. Blood cell $C^{14}$ analyses were mostly done in duplicate,

FIGURE 1




Concentration of $C^{14}$ in plasma and blood cell carbon following the feeding of labeled lysine to an anemic and hypoproteinemic dog.

FIGURE 2




Amount of $C^{14}$ circulating in the plasma and cell fractions of the blood. The approximate life span of the red cells is indicated.

intervals of about two months separating the duplicate determinations. The duplicate analyses gave reasonably corresponding results. The greatest difference found between duplicates was 18 per cent, the average difference, 5 per cent. The data for blood cells presented are an average of duplicate values.

Results: The urine specimen removed from the metabolism cage 8 hours after the administration of labeled lysine contained 27.8 per cent of the administered $C^{14}$. Total $C^{14}$ recovery from urine up to 72 hours totalled 34.9 per cent.

Figure 1 (Page 76) shows concentrations of $C^{14}$ per gm. of plasma carbon and per gm. of blood cell carbon following the feeding of labeled lysine. Experiments reported in detail (5) indicate that the plasma $C^{14}$ values for periods of 24 hours and longer after labeled lysine feeding represent predominantly radioactive carbon incorporated in plasma proteins.

The drop in $C^{14}$ concentration per gm. carbon in the blood cells (Figure 1, Page 76) in the 10 to 35 day period represents predominantly a dilution of labeled cells with new cells which the dog is able to form as the result of an adequate diet.

Figure 2 (Page 77) shows the total incorporation of $C^{14}$ in circulating cells and circulating plasma. The shape of curve shown is very similar to that resulting if the concentrations of $C^{14}$ in the cells and plasma of a unit quantity of blood are plotted as a function of time. In computing the curves of Figure 2 plasma values are based upon experimentally obtained plasma volumes. The value of circulating cells is estimated as 82 per cent of the value directly calculated from hematocrit and plasma volume determinations (2, 3). No direct data were obtained concerning the chemical form of the $C^{14}$ incorporated in the blood cells of this dog. In a rat the erythrocyte $C^{14}$ following feeding of this

tagged lysine was found almost exclusively in the globin portion of the isolated hemoglobin molecule.

Blood loss through sampling over the 234 days of this experiment represents about one-third of the dog's calculated 1000 ml. blood volume. No correction for this loss is made in computing the data for Figure 1 (Page 76) and Figure 2 (Page 77).

Discussion: Figures 1 and 2 demonstrate that this dog, depleted of both hemoglobin and plasma proteins, utilized the epsilon carbon of lysine with less lag in building plasma proteins than in building blood cells. Figure 2 shows that there is a turnover or utilization of newly formed plasma proteins as represented by a drop in $C^{14}$ content even under conditions where the animal is deficient in circulating plasma proteins and on an adequate diet is constantly increasing the total amount of plasma proteins in circulation.

Even when the total $C^{14}$ content of the plasma proteins is decreasing, the newly formed plasma proteins presumably may contain $C^{14}$ derived from labile body protein deposits, for example, liver. Therefore, the $C^{14}$ plasma disappearance curve can be used only to set a lower limit for the average rate of turnover of plasma proteins. As can be seen in Figure 2, the $C^{14}$ content of the plasma dropped from 4.4 to 2.2 per cent of the administered dose in a period of 5 days. On the assumption that this drop is logarithmic in nature, one can then compute that the average turnover of the plasma proteins incorporating $C^{14}$ occurs in less than 10 days. Corrections for blood loss do not significantly change this figure.

In contrast, as shown in Figure 2, the $C^{14}$ content of the pooled cellular elements of the blood increased much more slowly so that a peak value was achieved in 5 days. About 80 days after the time of administration of tagged lysine the

$C^{14}$ content of the blood cells began to fall rapidly. It reached a new plateau at about 155 days. This new level corresponds, as can be noted from Figure 1 (Page 76), to a $C^{14}$ content per gm. carbon approximately the same as the plasma level of the 60-150 day period. This suggests that the material initially formed in the blood cells from $C^{14}$ has been mostly removed during the period of 80-150 days and has been replaced from a pool with about the $C^{14}$ content of plasma protein over this period. As shown in Figure 2 (Page 77), about 110 days elapse between the point at which the $C^{14}$ content of blood cells achieves 50 per cent of its maximum value and the point where the $C^{14}$ content has decreased to a point midway between this maximum value and the plateau value beginning at 180 days. This period of 110 days clearly represents an approximation of the average time spent in red blood cells by $C^{14}$ atoms.

The above data are predominantly an indication of the fate of $C^{14}$ incorporated in the erythrocyte and show that the average time spent in erythrocytes by $C^{14}$ administered to this dog as DL-lysine epsilon carbon is about 110 days. One type of red cell removal was through blood sampling. A correction for such erythrocyte loss suggests that had no blood samples been taken, the above figure would be increased at least to 115 days.

The above figure of about 115 days is close enough to the erythrocyte life span of about 124 days for the average dog, as determined by Hawkins and Whipple (4), to suggest that what is actually being measured here is the life span of the dog's erythrocytes. The proteins of the erythrocytes of this dog are therefore predominantly fixed and stable entities which, subsequent to the formation of the circulating red cell, do not undergo metabolic reconstruction or turnover during the circulatory life span of the cell; at least this experiment gives no evidence of turnover. This finding is similar to the result reported

by Shemin and Rittenberg (8) in a study of hemin of the human erythrocyte carried out with $N^{15}$ used as a labeling element.

Some further deductions from the data obtained on this dog are plausible. The circulatory lives of the erythrocytes labeled in this dog are not homogeneous but fall predominantly in the range 80-150 days. The greatest rate of drop of $C^{14}$ content of circulating cells occurs over the period 110-130 days after the labeled amino acid feeding. This period of time probably corresponds to the period of greatest bile pigment excretion reported by Hawkins and Whipple (4) in their red cell life span experiments using bile fistual healthy dogs.

One further conclusion appears reasonable. The drop of $C^{14}$ concentration per gm. carbon in circulating cells to a value at 160 days close to the plasma value of the 60-120 day period suggests that the globin from destroyed cells is not used with great preference in the formation of new cells. Rather it seems that the amino acids for new hemoglobin formation are drawn from a much larger labile body store of which at any one time the plasma proteins with regard to $C^{14}$ content seem to be an adequate sample. This is in harmony with the observations (6) that show that in a fasting dog nitrogen balance can be closely or actually sustained by dog hemoglobin given intraperitoneally.

Summary: A dog, doubly depleted of blood cells and plasma proteins, was fed DL-lysine labeled with $C^{14}$ in the epsilon carbon position. In the first 8 hours 28 per cent of the administered $C^{14}$ was excreted in the urine; in the first 72 hours, 35 per cent.

Twenty-four hours after feeding, 4.2 per cent of the fed $C^{14}$ was circulating in the plasma, decreasing to 1 per cent at the end of 17 days. The $C^{14}$ content of the blood cells increased from 1 per cent at 24 hours to 5.5 per cent in 5 days and 6.8 per cent in 22 days.

Evidence based on the rate of decrease of the $C^{14}$ content of circulating blood cells is presented indicating an average life of 115 days for the erythrocyte protein as an entity not interchanging with extracellular constituents. This closely corresponds to the life span of the dog erythrocyte, 112-133 days according to the best evidence otherwise available and indicates that this experiment has actually measured the life span of the dog erythrocyte.

On the breakdown of blood erythrocytes the protein comprising them is not used preferentially for the formation of new erythrocytes.

## Bibliography


1. Bale, W. F., Manuscript in preparation.
2. Gibson 2nd, J. G., Peacock, W. C., Seligman, A. N., and Sack, T., J. Clin. Inv., 1946, 25, 838.
3. Hahn, P. F., Ross, J. F., Bale, W. F., Balfour, W. M., and Whipple, G. H., J. Exp. Med., 1942, 75, 221.
4. Hawkins, W. B., and Whipple, G. H., Am. J. Physiol., 1938, 122, 418.
5. Miller, L. L., Bale, W. F., Yuile, C. L., Masters, R. E., Tishkoff, G. H., and Whipple, G. H., J. Exp. Med., 1949.
6. Miller, L. L., Robscheit-Robbins, F. S., and Whipple, G. H., J. Exp. Med., 1945, 81, 405.
7. Olynyk, P., Camp, D. B., Griffith, A. M., Woislawski, S., and Helmkamp, R. W., J. Org. Chem., 1948, 13, 405.
8. Shemin, D., and Rittenberg, D., J. Biol. Chem., 1946, 166, 627.

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Problem Code: I.S.2 (Autoradiography)

Section Code: 3171

Author: George A. Boyd

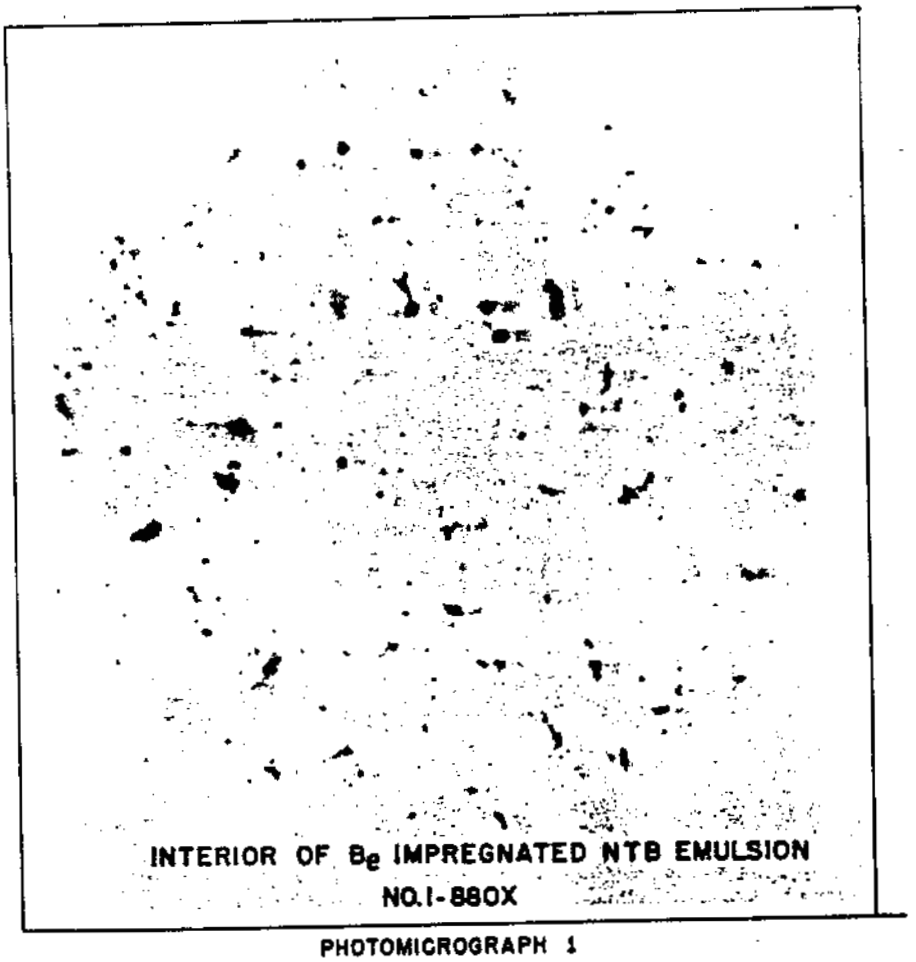
Autoradiographic Determination of Alpha-Emitting Contamination of Beryllium.

Background: It was suggested that this section use autoradiographic techniques to investigate the possibility of cosmic radiation producing intense localized ionization in beryllium. The question was raised, could such a process be a contributing factor in beryllium poisoning?

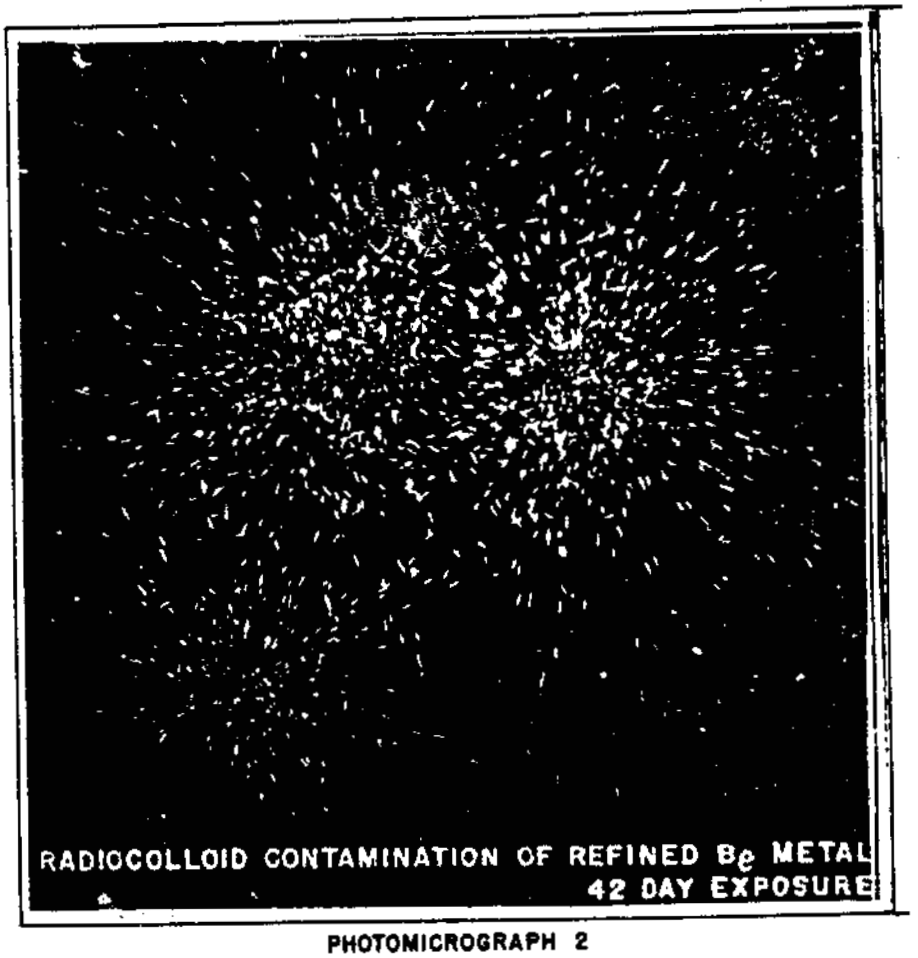
Method and Results: The Eastman Kodak Research Laboratories impregnated an NTB, 50 μ emulsion, #391,989, on September 30, 1948, with 0.4 grams of beryllium per $cm^2$. Plates were developed in D19 to bring out all nuclear tracks above mesons in specific ionization on the following dates:

| Dates of Development | Interval after Manufacture (in Days) |
|---|---|
| 12-8-48 | 69 |
| 12-28-48 | 89 |
| 12-28-48 | 89 |
| 1-10-49 | 102 |
| 1-10-49 | 102 |
| 4-11-49 | 223 |

No stars were found to indicate nuclear disintegration. Photomicrograph 1 (Page 84) is typical of the interior of the emulsion. The random clumps of silver grains might be interpreted as low voltage beta particles in non-impregnated emulsions. However, the presence of beryllium may in itself produce such grain-clumping. The clumps could also be interpreted as the tail end of a beta particle but again the above skepticism is in order. A test for beta tracks could be made




PHOTOMICROGRAPH 1




PHOTOMICROGRAPH 2

by impregnating NTB 3 plates which give tracks of beta particles up to 1 mev.

An independent approach was made to the question by making autographs of 7 square inches of rolled beryllium metal sheet, 0.012 inches thick; NTB plates #371,265 were used. The beryllium was furnished by Dr. Wolf and its history is unknown.

One square inch of the metal was pressed to the surface of the emulsion of each of seven plates. The area under the metal is the test area; the remainder of the plate is the control area. These were developed and the entire seven square inches were examined microscopically for random tracks, stars, and sunbursts. The results are given in Table 1 (Page 86).

Subtracting the mean of the control area, we have a mean of 30 alphas from the beryllium metal during the exposure time. Equal weight was given to the exposure times since greater accuracy is not indicated here. This is equivalent to 250 alphas/$cm^2$/exposure. Using the method of Finney and Evans (1) for calculating the radioactivity of solids, we found that one cc of beryllium produces 0.06 alphas per sec.

Discussion: The main question at issue in the experiment, viz., the possible presence of stars in photographic film as a consequence of cosmic ray bombardment of beryllium metal, must be answered in the negative for exposure times of up to 223 days. It would, therefore, appear unlikely that this reaction is a significant factor in beryllium poisoning.

A second problem introduced by the findings of this experiment is the identity of the elements giving rise to the single tracks and sunbursts found in the exposed photographic film. In order to explore this question, fifty tracks were measured in the control area and fifty in the test area. The results

TABLE 1

TRACKS, STARS, AND SUNBURSTS IN BERYLLIUM AUTOGRAPHS

| Autograph # | Random Alpha tracks per 100 fields* | | Stars** | Smeared Sunbursts*** per sq. in. | Autograph Exposure in days |
|---|---|---|---|---|---|
| | Control area | Test area Under Be | | | |
| 1 | 43 | 53 | 0 | 4 | 42 |
| 2 | 30 | 64 | 0 | 0 | 47 |
| 3 | 36 | 50 | 0 | 0 | 47 |
| 4 | 44 | 86 | 0 | 1 | 47 |
| 5 | 31 | 83 | 0 | 0 | 52 |
| 6 | 37 | 82 | 0 | 2 | 52 |
| 7 | 30 | 45 | 0 | 0 | 52 |
| Mean | 36 | 66 | | | |

*100 fields - 11.9 $mm^2$

** A star is a collection of tracks produced by nucleons of one or more species originating from a single nucleus.

*** A sunburst is a collection of tracks produced by nucleons of one or more species originating from a number of nuclei at one point.

are shown in Figure 1 (Page 88). Since the number measured was small, the observer was inexperienced, and the proper emulsion had not been used for accurate measurement, the distribution should not be considered too significant. It is probable that we have erred on the short side of the true length of the tracks since the emulsion was thin and tracks at all angles of the dip were measured and some of the alphas probably left the emulsion before all the energy was expended. Hence, the distribution should probably be shifted to the right.

If this is correct, the group at 20 $\mu$ for the test area may coincide with polonium. The half-life of polonium would indicate a much higher concentration at the earlier date of manufacture if polonium were to be considered as incorporated as such and not occurring as a disintegration product of other radioactive contaminants.

As an even more interesting finding was the presence of smeared sunbursts from point source contamination. An example is shown in Photomicrograph 2 (Page 91). The track lengths were measured. To improve on the accuracy, horizontal tracks only were measured. Thus tracks leaving the emulsions were not included. The histogram is shown in Figure 2 (Page 89).

Since $Th^{232}$ has the lowest energy alpha of the naturally occurring alpha-emitting elements, the distribution peaks at 8 and 12 u pose a question difficult to answer; viz., what is the source of the alphas?

It is not entirely certain that the tracks were produced by alpha particles. One track type is more fully developed than the other suggesting two types of nucleons. As the autoradiographs were not made for the purpose of differentiating between alpha, proton, and deuteron tracks, a decision was impossible. However, this could be done with a properly designed experiment. The heavy tracks accounted for about 45 per cent of the total; and the light,

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FIGURE 1

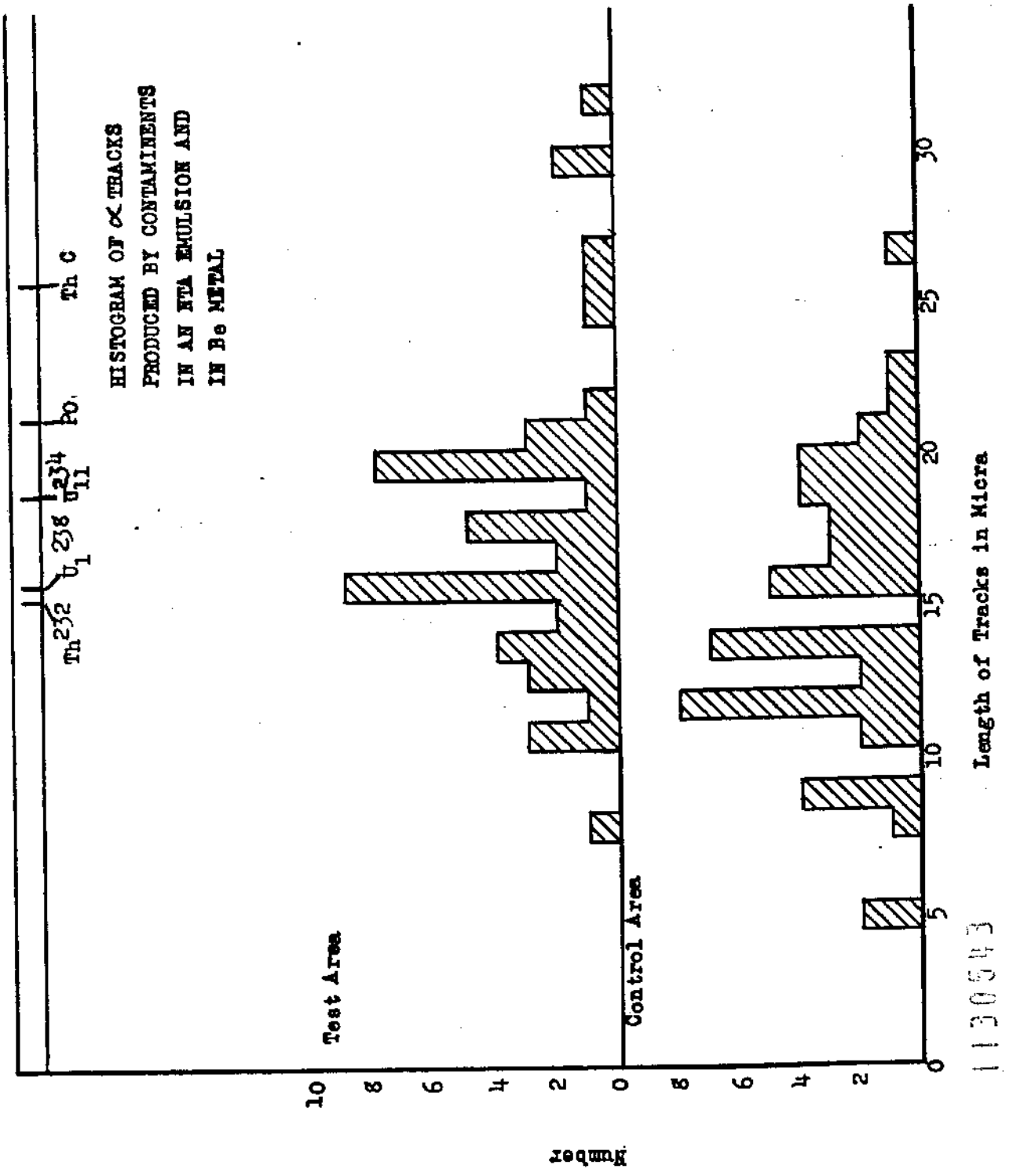
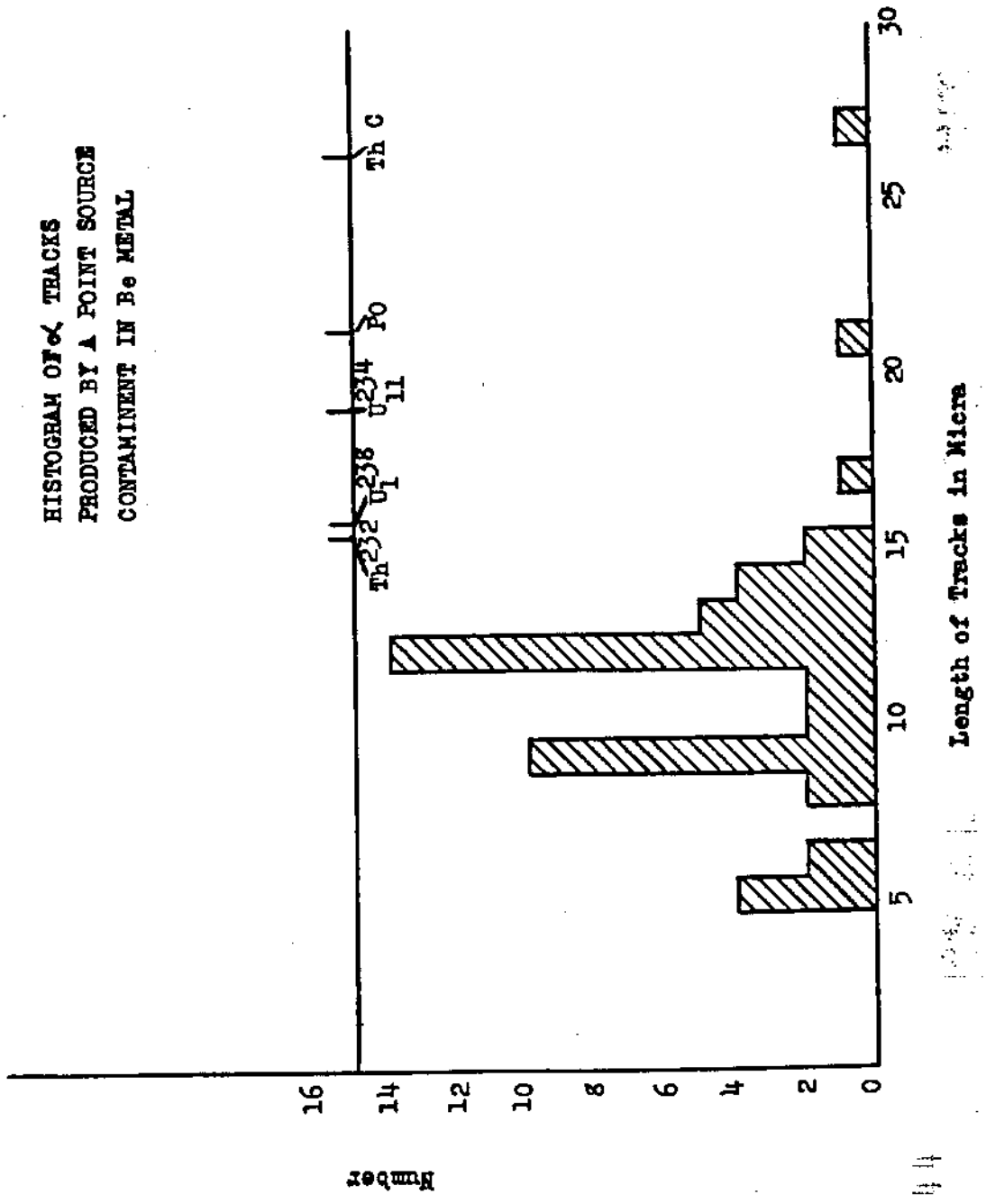


FIGURE 2



55 per cent.

If we assume the identity of the tracks, we can ascribe energy values to them as shown in Table 2 below.

TABLE 2

MEV OF PARTICLES FROM MEASURED TRACK LENGTH

| Assume | 8 μ | 12 μ |
|---|---|---|
| p | 0.75 | 0.85 |
| d | 1.50 | 1.70 |
| α | 2.25 | 3.2 |

A rather rapid survey of the literature gave no clue through known nuclear reactions of beryllium to account for any of the particle energies listed.

Nevertheless, the point sources of contamination exist and the following should be considered. Hevesy (2) and Hahn (3) have shown beryllium ores such as gadolinite to be contaminated with alpha emitters and have considered this as a source of neutrons. Yagoda (4) showed by autoradiography that a beryl crystal contained alpha contamination in both random form and in point sources.

Bibliography

1. Gladys D. Finney and Robley D. Evans: "The Radioactivity of Solids Determined by Alpha-Ray Counting", Physics Review, 48, 502, 1935.

2. G. von Hevesy and H. Levi, Proc. Copenhagen Roy. Soc., 14, 25, 1936.

3. O. Hahn, Cited as a personal communication by I. Oftedal, Norsk Geol. Tids., 19, 341, 1940.

4. Herman Yagoda, Radioactive Measurements with Nuclear Emulsions, John Wiley & Sons, 1949.

Problem Code: I.S.3 (Therapy)

Section Code: 3340

Author: Kenneth P. McConnell

Background: Several isotopes of selenium, both stable and radioactive, are produced which yield from .008% to .21% (1). In view of the fact that relatively little information is available about the metabolism of trace amounts of selenium in the mammalian organism, it was felt that studies on the behavior of selenium in the body would prove to be of value in our better understanding of selenium metabolism.

One phase of current interest in the selenium problem is that selenium like certain other elements has an affinity for proteins. The mechanism by which the fixation of selenium takes place in proteins is at present not clearly understood. A possible hypothesis is that selenium displaces the amino acid sulfur in some form such as selenocystine, or actually binds sulfhydryl groups.

It was the plan of the experiments presented here to study the qualitative and quantitative distribution of selenium in the various blood proteins and red blood cells after injecting sodium selenate carrying trace amounts of radio-selenium.

Method: Four normal dogs weighing from 7.3 to 9.1 Kg. were injected subcutaneously three times at 24-hour intervals with subtoxic amounts of sodium selenate containing radio-selenium. Previous to the second and third injections,

and 24 hours after the last injections, samples of blood were taken from the carotid artery. Hematocrit and routine hematological studies were made. The red cells and plasma were separated by centrifugation, and the cells washed twice with isontonic saline. Radioactivity measurements were made on samples of whole blood, plasma, and packed red blood cells, all of which were measured on a bell-type Geinger-Muller tube. Selenium-75 has a half life of 115 ± 5 days which decays through K electron capture, emitting a probable .4 M.E.V. gamma ray with the formation of stable $As^{75}_{33}$.

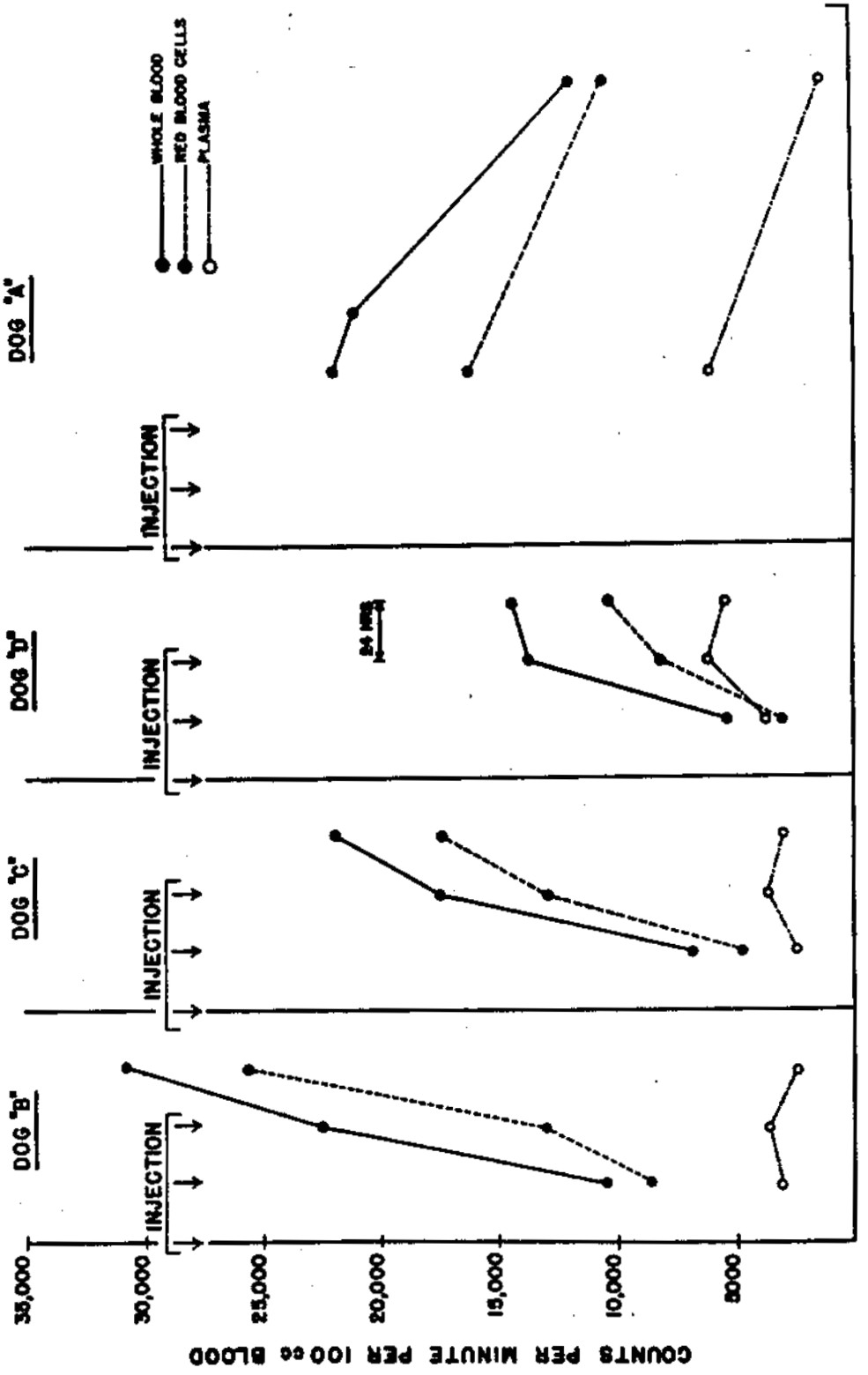
In the experiments to study the distribution of selenium in the various serum proteins, samples of blood were taken 24 hours after the last injection. Total proteins were precipitated with a.5% trichloroacetic acid. Fractionation of the various serum proteins was carried out using the method of Majoor (2) as modified by Milne (3). The protein precipitates were analyzed for radioselenium, and the protein content of the precipitates and filtrates were determined by Clark's micro-Kjeldahl method (4).

Crystalline hemoglobin was isolated from the red blood cells of three dogs that had been injected with sodium selenate containing radioselenium, using the method of Welker and Williamson (5). Crystalline hemin was isolated by the method of Nenki and Zaleski (6). The globin fraction was studied following hydrolysis of crystalline hemoglobin with acetone and hydrochloric acid after the method of Anson and Mirsky (7). The method of Grinstein was used to isolate protoporphyrin from red blood cells.

Results: It was observed that the concentration of selenium in whole blood and red blood cells increases appreciably with time, and that the concentration of selenium is greater in the red blood cells than in the plasma (Figure 1, Page 93). It appears that the concentration of selenium in the plasma, when

FIGURE 1

DISTRIBUTION OF SELENIUM IN DOG PLASMA AND RED BLOOD CELLS AFTER REPEATED SUBCUTANEOUS INJECTION OF SODIUM SELENATE CONTAINING RADIOSELENIUM



determined at 24 hour intervals, varies within very narrow limits throughout the experiment. The per cent of whole blood activity in red blood cells for four dogs averaged 79% (72% to 91%), while the per cent of activity in plasma averaged 22% (8% to 39%). It was calculated that 2.0% to 3.9% of the selenium administered was present in the total circulating blood.

In the experiments on the distribution of selenium in the various protein fractions it was found that selenium was present in albumin, globulin, euglobulin and pseudoglobulin, and that the amount of selenium in the various fractions when expressed as gamma selenium per gram protein was similar but not identical. It appears that the selenium fixation takes place to about the same degree in the globulin and albumin, for the percentages of globulin and albumin selenium in total protein selenium for three dogs was found to be 54 ± 10% and 46 ± 10% respectively.

In the study on the distribution of selenium in the red blood cells it was found that trace amounts of selenium were present in crystalline hemoglobin and hemin, and in globin. The amounts of selenium in hemin when calculated on the gram basis are greater than in globin. Isolation and analysis of protoporphyrin from radioactive red blood cells showed the presence of radio-selenium.

## Bibliography

1. Plutonium Project Report, Jr. Am. Chem. Soc., 68, 2411, 1946.

2. Majoor, C. L. H., Thesis, Amsterdam (1942); Yale J. Biol. and Med., 18, 419, 1946.

3. Milne, J., J. Biol. Chem., 169, 595, 1947.

4. Clark, E. P., J. Assoc. Offical Ag. Chem., 24, 641, 1941.

5. Welker, W. H., and Williamson, C. J., J. Biol. Chem., 41, 75, 1920.

6. Nenki, M., Zaleski, J., Z. Physicol. Chem., 30, 384, 1900.

7. Anson, M. L., and Mirsky, A. E., J. Gen. Physiol., 13, 469, 1930.

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PROGRAM O.S.

OUTSIDE SERVICES

Problem Code: None

Section Code: 3150

Reported By: L. T. Steadman

Spectrographic Service Analyses

1. 524 air dust samples were analyzed for beryllium.
2. 6 standard samples were analyzed for beryllium.
3. 1 skin biopsy specimen was analyzed for beryllium.

Problem Code: None

Section Code: 3310

Reported By: Russell Hayes

The following services were rendered:

a) 733 badge monitoring films processed.

b) 45 radon atmospheric analyses (Dr. Hursh).

c) 524 beryllium dust analyses (Dr. Steadman).

d) 315 fingerprint analyses (Mr. Hay).

e) 1 tissue analyses for Be (Dr. Steadman).

In addition to receiving and distributing the above samples, and collecting and reporting these samples, all incoming and outgoing isotopes for the project were ordered and received through our department.

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PROGRAM I.N.

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

Problem Code: I.N.2 (Service)

Section Code: 3150

Reported By: L. T. Steadman

Spectrographic Service Analysis

1. 50 chamber dust samples were analyzed for beryllium.
2. 391 animal tissues were analyzed for beryllium.
3. 42 special solutions were analyzed for beryllium.
4. 20 special samples were analyzed for sodium.
5. 6 samples were analyzed for various other elements.

## TECHNICAL REPORTS ISSUED FOR DISTRIBUTION

April 1, 1949 thru June 30, 1949

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