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UR-38

Health and Biology - General

THE UNIVERSITY OF ROCHESTER
Atomic Energy Project
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QUARTERLY TECHNICAL REPORT

April 1, 1948 to June 30, 1948

Submitted by: Henry A. Blair,
Director

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Columbia University (Failla)	31
General Electric Company	32-35
Hanford Directed Operations	36-42
Iowa State College	43
Kellex Corporation	44-45
Los Alamos	46-48
Massachusetts Institute of Technology	49
Monsanto Chemical Company, Dayton	50-51
National Bureau of Standards	52
Naval Radiation Laboratory	53
NEPA	54
New York Directed Operations	55-57
Oak Ridge National Laboratory	58-69
Patent Advisor, Washington	70
Technical Information Division, ORDC	71-85
UCLA Medical Research Laboratory (Warren)	86
University of California Radiation Laboratory	87-91
University of Rochester	92-93
Western Reserve University (Friedell)	94-97

Internal Distribution (Rochester)	
Library	98-99
Dr. Dowdy	100
Dr. Blair	101
Dr. Bale	102-103
Dr. Hodge	104-105
Dr. Howland	106-107

TABLE OF CONTENTS

INTRODUCTION

The scientific work presented herein has been coded at the program and problem levels according to the scheme given on Pages 6 and 7. In the report all contributions to a given problem have been assembled together without regard to author or 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 1 what administrative officer can be approached for information about particular work. This does not imply either authorship, or scientific credit which will appear only in final reports issued from this Project. Since only progress in specific scientific problems is being reported herein, the cumulative work of special service units is not given separately. Their contributions appear or are implied in the reports on problems in which they participated.

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.

EXPLANATION OF PROGRAM AND PROBLEM CODES

The scientific work of 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 into problems was possible, an attempt was made to achieve a natural division in the sense that each problem would encompass a subject normally written up and generally considered as a unit. The program on chemical toxicity of uranium, for example, has been broken down into problems according to the divisions commonly employed by toxicologists.

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

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

PROGRAM AND PROBLEM CODES

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

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

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

- I.R.1 Flash Burns

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

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

IV. U. URANIUM

- U.1 Physical and Chemical Properties
- U.2 Toxic Effects (description of acute and chronic toxicity)
- U.3 Toxic Limits (respiratory; oral; skin; eye; parenteral)
- U.4 Fate (distribution and excretion)

IV. U. URANIUM (cont.)

U.5 Mechanism of Toxic Effects

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

V. Be. BERYLLIUM

Be.1 Physical and Chemical Properties

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

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

Be.4 Fate (distribution and excretion)

Be.5 Mechanism of Toxic Effect

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 Effect

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

ORGANIZATION

I. DIVISION OF RADIOLOGY AND BIOPHYSICS (3100): William F. Bale

Section Code	Section	Administrative Head
3110	Instrumentation	John B. Hursh
3120	Tracer Chemistry	Leon L. Miller
3130	Radiation Physiology	Thomas B. Noonan
3133	Radiation Animals	Thomas B. Noonan
3134	Autoradiography	Thomas B. Noonan
3140	Radiation Chemistry	Kurt Salomon
3150	Spectroscopy	Luville T. Steadman
3160	Radiation Mechanics	Francis W. Bishop
3161	Electron Microscope	Francis W. Bishop
3170	Radiation Toxicology	J. Newell Stannard

II. DIVISION OF PHARMACOLOGY AND TOXICOLOGY (3200): Harold C. Hodge

Section Code	Section	Administrative Head
3210	Industrial Hygiene	Herbert E. Stokinger
3220	Biochemistry	William F. Neuman
3230	Ingestion Toxicity	Elliott Maynard
3250	Pathology	James K. Scott
3260	Physiology	Aser Rothstein

III. DIVISION OF MEDICAL SERVICES (3300): Joe W. Howland

Section Head	Section	Administrative Head
3310	Industrial Services	J. Russell Hayes

III. DIVISION OF MEDICAL SERVICES (3300): Joe W. Howland (cont.)

Section Code	Section	Administrative Head
3312	Clinical Problems	Joe W. Howland
3320	Health Physics	Herbert E. Hermagen
3330	Project Medical Service	Joe W. Howland
3340	Medical Research	Joe W. Howland
3390	Photographic Unit	Joe W. Howland

IV. DIVISION OF DIVERSIFIED PROBLEMS (3400): Henry A. Blair

Section Code	Section	Administrative Head
3410	Mouse Genetics	Donald R. Charles
3420	Hematology	William N. Valentine
3430	Surgery	Paul E. Rekers
3440	Protein Metabolism	G. Burroughs Mider
3441	Embryology	Karl E. Mason James G. Wilson
3442	Immunity	William L. Bradford
3450	Flash Burns	Herman E. Pearse
3460	Theoretical Problems	W. Burkett Mason

PROGRAM X.R.

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

Problem Code: X.R.1 (Tolerance Studies)

Section Code: 3130, 3133

Pathological Findings in Pigeons Subjected to a Single Dose (5,000 r) of Total Body X-Radiation.

Background: Previous work with pigeons has indicated that this animal possesses a relatively high resistance to x-radiation. For this reason it became of interest to obtain information concerning the pathological effects of x-radiation on various organs. To this end the following preliminary experiment was carried out.

Method: Eleven pigeons, eight females and three males, were used. Two females and one male were retained as controls. Six females and two males were subjected singly to an acute dose of total body x-radiation (5,000 r; 250 K.V.; 15 m.a.) given at the rate of 25 r per minute and with a target skin distance of 15 inches. The filter was aluminum parabolic plus $\frac{1}{2}$ mm. of copper.

Six of the radiated birds were sacrificed singly by decapitation at 1, 2, 3, 4, 5, and 7 days after radiation. The one sacrificed on the 7th day was near death. Two others died approximately $7\frac{1}{2}$ days after radiation. One was suitable for pathological study and the other was discarded because of extreme post-mortem autolytic changes. The control pigeons were sacrificed singly at various times during the series.

The organ samples taken at autopsy for histopathological examination were fixed in Bouin's solution, stained with Harris' hematoxylin and eosin.

and included samples of thyroid, heart, lung, liver, pancreas, spleen, stomach (proventriculus and gizzard), small intestine, large intestine, kidney, gonads (testes or ovaries), oviduct, skin, femoral bone marrow, and femur.

Results: No definite and significant changes were found in the lungs, heart, skin, ovaries, oviduct, gizzard, and bone.

Minimal changes, consisting of degeneration of very small numbers of parenchymatous cells, were found in the thyroid, pancreas, kidney, and proventriculus of most of the radiated pigeons. In the kidney of the bird sacrificed on the 4th post-radiation day, however, degeneration of cortical tubular and glomerular epithelium was moderately marked, necrosis of epithelium was prominent, considerable regenerated epithelium was apparent, hyaline changes were present in glomeruli, and some arterioles were occluded by swollen endothelial cells. Although these renal changes are compatible with the effects of irradiation, it is thought that some other factor may have influenced the advanced degree of the lesion in this one bird.

Compared with the thyroid of other laboratory animals, such as the mouse, rat, cat, rabbit, dog, and monkey, the thyroid of the pigeon generally contains very little colloid in the acinous [illegible] and is often in an hyperplastic condition.

Hyperossification, or formation of cancellous bone, associated with the reproduction cycle of the female pigeon, was found in three cases.

Hepatic changes consisted chiefly of increased cytoplasmic vacuolization (apparently fatty metamorphosis) of hepatic cells in radiated pigeons. These vacuoles were small in number in one control bird and in the radiated bird which was sacrificed on the 2nd post-radiation day. The vacuoles were

moderately marked in number in the radiated pigeons autopsied 5, 7, and 7½ days after radiation, at which times small numbers of hepatic cells with degenerate nuclei were also found. Only very small amounts of blood pigment were observed in control and radiated livers.

Major pathological changes were found in the intestines, spleen, bone marrow, and testes.

The changes in the small and large intestine were essentially of the same type but were generally more marked in the small intestine. On the 1st post-radiation day considerable decrease in the number of mitotic figures was apparent. Depression of mitosis was marked on the 2nd day and some of the gland cells were enlarged and contained vesicular nuclei. On the 3rd, 4th, and 5th days mitoses were rare, the large and vesicular nuclei were more numerous and more widespread in the glands, considerable numbers of epithelial cells were frankly degenerate, and some were necrotic. At these times cystic glands were prominent in the small intestine. These cystic glands were dilated and lined with low, flattened epithelium.

The appearance of the small intestine after this time suggested regenerative activity. On the 7th post-radiation day mitoses were present in moderate numbers, only small numbers of large vesicular nuclei were present in the glands, and cystic glands were reduced in number. In the large intestine, however, mitotic figures were still rare, large and vesicular nuclei were fairly numerous in the glands, and degenerate and necrotic epithelial cells persisted. The pigeon which died 7½ days after radiation revealed further regeneration in the small intestine and some indication of regeneration in the large intestine as well. Mitotic figures were present in moderate numbers, degenerate and necrotic epithelial cells were rare, only small

numbers of epithelial cells with large and vesicular nuclei remained, and in the small intestine no cystic glands were found.

The spleens of radiated pigeons revealed hypoplasia and reduction in size, as compared with controls. In the 1st post-radiation day hypoplasia or loss of functional cells was slight. On the 2nd day hypoplasia was marked and reduction in the size of the spleen was moderately marked. This was generally the condition of the spleen during the remainder of the series. Very little necrotic cellular debris was evident in the spleen of any pigeon examined. The altered blood pigment, hemosiderin, so commonly seen in the spleen of rats and other laboratory mammals, especially after large doses of x-ray, was conspicuously absent in the spleens of all pigeons in this experiment.

The bone marrow revealed the most marked change of any of the organs examined. Hypoplasia was slight on the 1st post-radiation day, mild on the 2nd day, moderately marked on the 3rd day, and very marked on the 4th and 5th days. The pigeon sacrificed on the 7th day and that which died 7½ days after radiation revealed almost extreme hypoplasia of the bone marrow. The parenchymatous elements of the marrow had been replaced by fat. Very little necrotic cellular debris was found in the marrow of any of the pigeons examined.

The testes of the radiated male pigeons revealed marked changes, as compared with those of the control males. In the control there were small numbers of degenerate spermatogenic cells, including some giant multinuclear cells formed by the coalescing of degenerate spermatids. In the radiated male which was sacrificed on the 2nd post-radiation day spermatogonia were rare, spermatocytes were reduced in number to a moderate degree, and

considerable numbers of degenerate spermatogenic cells were found in the seminiferous tubules. The male pigeon which died 7½ days after radiation revealed extreme decrease in the number of spermatogonia, marked losses of spermatocytes and spermatids, and moderate decreases in number of spermatozoa in the seminiferous tubules. Many of the spermatogenic cells which remained in the tubules were degenerate.

Discussion: Marked pathological changes, compatible with the effect of radiation, have been produced in the intestines, spleen, bone marrow, and testes of pigeons subjected to 5,000 r acute total body x-radiation.

Fat vacuoles were moderately increased in the livers of radiated pigeons as compared with controls.

Degenerative changes were found in the nuclei of small numbers of parenchymatous cells of the liver, thyroid, pancreas, kidney, and proventriculus of most of the radiated pigeons. In one case degenerative change in the kidney was extensive.

An experiment which was carried out in this laboratory, in which adult albino rats were subjected to 800 r acute total body x-radiation and were studied pathologically over a period of 10 days, is most convenient at present for a comparison of species with respect to x-radiation. The survival time of these rats, 8 to 12 days, was not very different from that of the pigeons in the present experiment. The major pathological findings in the present experiment, involving the intestines, spleen, bone marrow, and testes, were essentially the principal findings in the rat experiment, and the degrees of these changes in the two experiments were not markedly different. Intestinal and testicular changes were somewhat greater in the pigeons than in the rats. In both rats and pigeons considerable repair and

regeneration occurred in the intestines before death occurred. The rats, however, did not reveal the vacuolization in the liver and the minimal degenerative changes in the thyroid, pancreas, kidney, and glandular stomach.

The order of organ sensitivity in the pigeon, according to the present data, appears to be roughly the same as that found in the rats, at least in a consideration of the intestines, spleen, bone marrow, and testes. The data, however, are as yet too meager for definite conclusions in this matter.

Although the major pathological changes in the present pigeons and in the rats cited are reasonably similar in degree, the difference in dosage is more than six-fold. A slightly higher dose in the case of the rats would tend to make the degrees of pathological change in various organs generally more similar to those in the present experiment and to reduce the survival time of the rats to the survival time range of the pigeons receiving 5,000 r. If one were to hazard an estimate of the relative or comparative resistance of the pigeon to x-radiation on the basis of these considerations, it would appear that the pigeon is probably about five times as resistant to x-radiation as the adult albino rat. More data are needed, however, at various dosage levels for an accurate appraisal in this matter.

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

Section Code: 3140

Some Aspects of Glycine Metabolism. I. Experiments with Bone Marrow in Vitro (Studies on Porphyrin and Fatty Acid Metabolism).

Background: It has been shown in this laboratory by in vivo experiments with C^{14} that the alpha carbon atom of glycine is incorporated in the

hemin molecule (1). As an extension of this work, the utilization of glycine by bone marrow homogenates is being studied. The present report is concerned with some results of this study.

In addition to the in vitro incorporation of the alpha carbon of glycine into the hemin molecule, the experiments show that fatty acids isolated from the homogenate contain very considerable amounts of activity after incubation of the medium with added C^{14} marked glycine. This is noteworthy since (a) evidence from feeding experiments (2) (3) utilizing tagged compounds indicates that glycine is not converted to acetate, and (b) acetate is considered the obligatory precursor of fatty acids.

Method: Preparation of bone marrow homogenates: The marrow of the long bones of rabbits was removed from the bony cavity by a blast of air and then suspended in bone marrow Ringer as described by Needham and collaborators (4). The marrow was then transferred to a Waring blender cup and homogenized. Approximately 5 cc. of Ringer were used for one gram of bone marrow wet weight, and the homogenate was incubated at 37°C for varying lengths of time. After incubation of the bone marrow suspensions for various periods of time, hemin was isolated and crystallized. Since it is conceivable that the hemin obtained is contaminated by a high activity contaminant, degradation of hemin to protoporphyrin dimethylester was carried out in order to assure

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(1) Altman, K. I., Gosarett, G. W., Masters, R. E., Noonan, T. R., and Salomon, K. Fed. Proc., 7:2 (1948).

(2) Sonne, J. C., Buchanan, J. M., and Delluva, A. M. J. Biol. Chem., 173:69 (1948).

(3) Buchanan, J. M., Sonne, J. C., and Delluva, A. M. J. Biol. Chem., 173:81 (1948).

(4) Needham et al. Biochem. J., 41:631 (1947).

the chemical purity of the compound isolated. In all the experiments reported the dimethylester was recrystallized twice before the measurement of activity. The protoporphyrin dimethylester is recrystallized with ease and has a well-defined melting point, assuring a product of high chemical purity. The method of M. Grinstein was used (5). Fats were extracted with ether alcohol mixtures (2-1), saponified, and then separated into saturated and unsaturated fractions on the basis of their lead salts. The lead salts were then decomposed and the acids converted to their methylesters.

Results:

1. Protoporphyrin Synthesis: Table I (Page 21) shows the results of several experiments and lists the activity per gram protoporphyrin as a function of different incubation times.

2. Fat Metabolism: Table II (Page 22) lists the activity measured for the saturated and unsaturated fatty acid fractions after incubation for varying lengths of time.

Discussion: These experiments demonstrate that cell-free bone marrow homogenates are capable of utilizing the alpha carbon of glycine in the formation of (1) hemin and (2) fatty acids.

The metabolism of bone marrow is being studied in detail in order to lay the basis for an investigation of the mechanism of radiation injury as it affects the hemopoietic system.

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(5) Grinstein, M. J. Biol. Chem., 157:515 (1947).

TABLE I

PROTOPORPHYRIN SYNTHESIS IN BONE MARROW HOMOGENATES

Time Hrs.	C^{14} activity of protoporphyrin dimethylester in units x 10⁴ disint./min/gm.
1/2	0.0
1 1/2	0.0
3	36.7
17 1/2	77.0

TABLE II

INCORPORATION OF THE α-CARBON ATOM

OF GLYCINE IN BONE MARROW FATS

Time of Incubation Hrs.	C^{14}-activity of saturated fatty acid methyl esters units x 10^4 disint./min/gm	C^{14}-activity of unsaturated fatty acid methyl esters units x 10^4 disint./min/gm
16	0.148	0.046
25	0.129	0.046
67	0.563	0.594

Some Aspects of Glycine Metabolism. II. Experiments with Spleen Homogenates In Vitro.

Background: In certain species (e.g. the rabbit) histological evidence points to the ability of spleen tissue to carry out erythropoiesis. Therefore, it seemed of interest to investigate chemically hemoglobin synthesis in spleen tissue.

Method: Rabbit spleen homogenates were used and incubated at 37°C in the presence of alpha carbon labeled glycine. Hemin was isolated from the incubation mixture by the usual procedure.

Results: The results are tabulated in Table III (Page 24).

Discussion: The experiments reported demonstrated that the rabbit spleen homogenates are able to synthesize hemin. This study is a part of a program to investigate the biochemical mechanism underlying radiation effects on the blood forming organs.

TABLE III

HEMIN SYNTHESIS IN SPLEEN HOMOGENATES

Time of Incubation Hrs.	C^{14}-activity of hemin in units x 10^4 disint./min./gm.
$15\frac{1}{2}$	27.3
25	91.2

Problem Code: X.R.3 (Therapy)

Section Code: 3430

Rutin Studies:

The intravenous administration of large quantities of heparin to normal dogs results in an immediate but transient thrombocytopenia without significant hemoconcentration, hemodilution or alteration of the sedimentation rate.

When heparin is inactivated by acid hydrolysis, a similar thrombocytopenia is not produced. Large quantities of histamine, likewise, are without effect on the thrombocyte level.

In vitro studies indicate that the sedimentation rate of a dog's blood is increased by heparin. However, this sedimentation rate change is counteracted by the addition of rutin in $NaHCO_3$. Hesperidin methyl chalcone and homoeriodictyol, related flavonones, do not have the same inhibiting effect as rutin in vitro.

When rutin and heparin are applied in vivo, only a thrombocytopenia is noted, an effect found with heparin alone.

Data from such experiments are recorded in the accompanying Tables 1 and 2 (Pages 26 and 27).

TABLE 1

EFFECT OF HEPARIN AND HISTAMINE ON THROMBOCYTES

Time	Heparin			Inactivated Heparin			Histamine			Heparin & Histamine			Heparin & Rutin		
	Platelets* 100,000/mm^3	Hct. %	Sed. Rate mm/hr.	Platelets* 100,000/mm^3	Hct. %	Sed. Rate mm/hr.	Platelets* 100,000/mm^3	Hct. %	Sed. Rate mm/hr.	Platelets* 100,000/mm^3	Hct. %	Sed. Rate mm/hr.	Platelets* 100,000/mm^3	Hct. %	Sed. Rate mm/hr.
Pre-injection	1.6	46	03	2.3	43	01	2.3	40	16	3.3	45	00	2.4	46	08
5' Post-injection 1	0.2	48	02	0.8			2.1			0.2			0.0		
15'	0.1	49	02	2.1			2.2			0.2			0.2		
30'	0.1	49	02	2.2			2.0			0.4			0.4		
60'	0.4	46	03	2.4	42	01	2.0	46	13	1.2	46	00	1.0	43	06
120'	1.1	44	03	1.8	40	02	1.7	42	07	2.2	46	00	2.0	43	07
5' Post-injection 2	0.1	45	02	1.3			2.2			0.5			0.1		
15'	0.2	50	01	1.7			2.3			0.6			0.4		
30'	0.3	51	01	2.2			1.8			0.8			0.6		
60'	0.9	42	03	1.8	39	02	1.6	45	09	1.3	44	00	0.5	39	[illegible]
120'	1.1	42	04	2.3	40	02	1.7	41	07	1.8	44	00	1.7	38	[illegible]
5' Post-injection 3	0.2	40	02	1.8			2.5			0.2			0.3		
15'	0.7	40	03	1.5			2.4			0.8			0.4		
30'	0.3	42	07	2.5			2.5			1.5			0.3		
60'	0.6	39	05	2.5	40	01	1.9	40	02	1.6	41	01	1.4	38	[illegible]
120'	1.1	43	02	2.5			1.2			2.1			1.1		

* Determinations carried out in duplicate.

TABLE 2

EFFECT OF VARIOUS AGENTS ON SEDIMENTATION RATES

	Supplements to 2 ml oxalated dog blood	Corrected sedimentation rate mm/hr
Test A.	1. plus 0.2 ml saline solution (control)	0
	2. plus 1 mg Histamine	0
	3. plus 10 mg Histamine	0 (hemolysed)
	4. plus 1 mg Heparin	2
	5. plus 10 mg Heparin	25
	6. plus 10 mg Hyaluronidase	1
	7. plus 20 mg Hyaluronidase	3
Test B.	1. plus 0.2 ml saline plus 0.2 ml 1% $NaHCO_3$ (control)	0
	2. #1 plus 10 mg Heaprin	24
	3. #2 plus 0.2 mg Rutin	12
	4. #2 plus 2 mg Rutin	0
	5. #2 plus 2 mg Hesperidin methyl chalcone	0
	6. #2 plus 2 mg Homoeriodictyol*	12
	7. #2 plus 2 mg Hesperidin*	20
	8. #1 plus 5 mg Heparin	7
	9. #8 plus 0.2 mg Rutin	3

The flavanones were dissolved in 1% $NaHCO_3$ solution.
* These substances did not readily dissolve in the $NaHCO_3$ solution.

PROGRAM U.

URANIUM

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

Section Code: 3210, 3220

Dispersion of Aerosols:

Submerged Aerosol Unit: Considerable progress has been made in the development of simplified stainless-steel aerosol units of the submerged type previously described. The latest form of this instrument is illustrated in Figure 1 (Page 29). The dispersal jet consists essentially of a 2-part construction using 0.027" orifices for both the liquid and air jets. The overall diameter is 9/16" and the working height 23/32". Similar to this previously described instrument, this unit can be operated from either submerged or elevated positions. Preliminary experiments with its use as a submerged instrument indicate that variation in the head of liquid above the jets will permit the production of a wide range of particle sizes. A one liter round-bottomed flask as illustrated was found to be the most successful container tube. For portable operation, the low-cost Gast* compressor unit which is self-contained with $\frac{1}{4}$ horsepower motor was found to be highly successful in operation over a range of pressures up to 20 pounds.

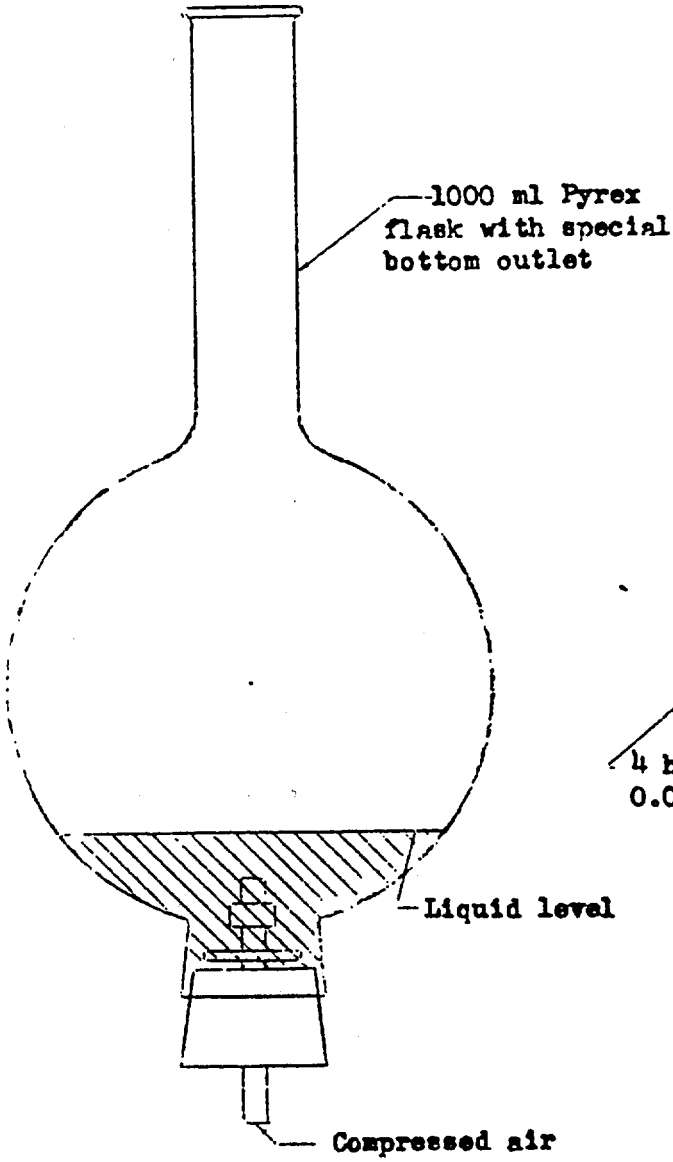
Studies are continuing on the efficiency characteristics of these aerosol units.

The Isolation of Microquantities of Uranium from Solution by Means of Protein - A Study of the Factors Involved:

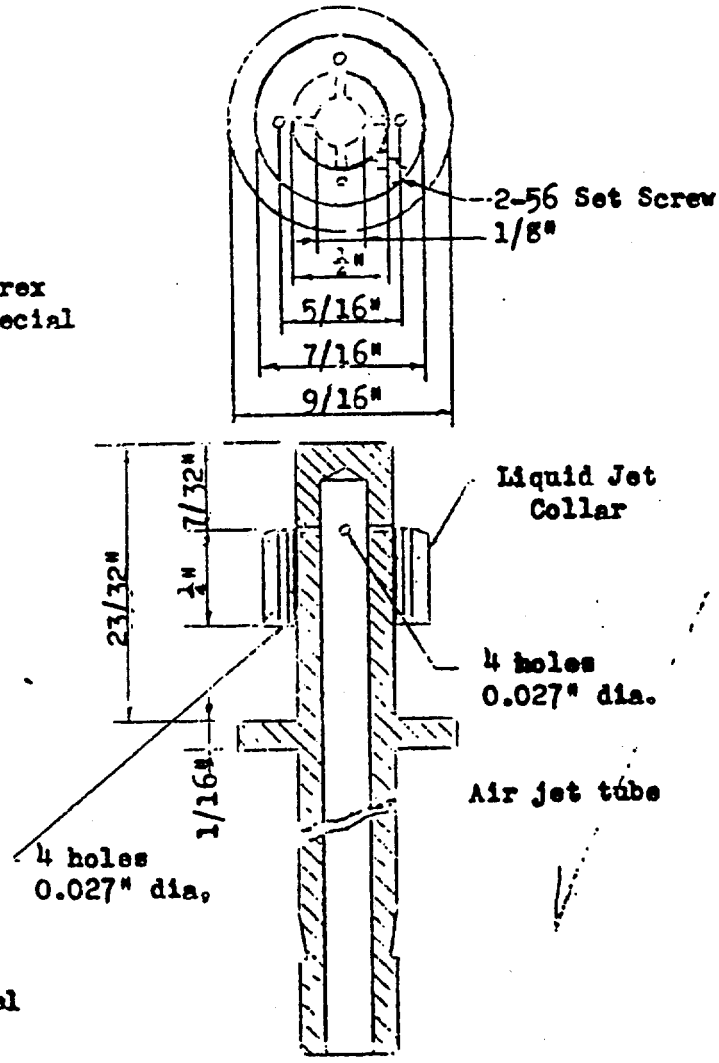
A report in preparation for publication presents a detailed study of

* Gast Mfg. Co., Benton, Michigan

SUBMERGED AEROSOL UNIT



Assembled Apparatus
½ Actual Size



Four Jet Aerosol Unit
Stainless Steel
Twice Actual Size

Sidney Laskin 6/1/43

the conditions required for the quantitative separation of uranium by means of protein from substances which interfere with its fluorometric determination. This separation was prerequisite to the fluorometric determination of uranium and has been used in studies on the distribution and excretion of uranium in animals.

The general procedure was as follows: the acid solution of ashed tissue and uranium was neutralized with alkali to pH 4.2-5.0, made 0.05 molar with acetate buffer, pH 5.0, 120 milligrams of albumin then added, and the solution volume adjusted to 35 ml. with distilled water. After heating the solution, the coagulated protein-uranium mass was centrifuged, washed once with 0.05 molar acetate buffer, dissolved in concentrated nitric acid, and analyzed fluorophotometrically. Quantities of uranium as large as 2-5 mg. and as small as 10 μg or less may be isolated quantitatively from 35 ml. of pure solution under such conditions. Within this concentration range, the pH is not critical in the vicinity of the isoelectric point of the albumin (pH 4.4-5.5). Increasing the protein concentration, the pH, or decreasing the buffer concentration will raise this upper limit of uranium concentration which may be quantitatively isolated.

The inverse relationship between buffer concentration and percentage of uranium isolated has been established and its importance demonstrated. All of the readily available buffers react with uranium and thus compete with the protein. Acetate buffer, being the weakest complexer, was chosen for the procedure. No difference was observed between sodium and ammonium acetate. Citrate has 10 or more times the complexing affinity for uranium as acetate at pH 4.6 or higher. Because over 90 per cent of the uranium can be removed with bicarbonate, it is concluded that the protein-uranium combination is dissociable even though the protein is coagulated.

Quantitative isolation of uranium from solution was obtained with crystallized egg albumin, bovine serum albumin, Fraction V from bovine plasma, and commercial preparations of egg albumin powders.

Quantitative isolation of uranium was achieved from solutions of ashed kidney and bone at ratios of 1:100 and 1:1000 of uranium: tissue ash. The bone studies using Ca^{45} showed that at pH 5.0 the isolated uranium was practically free of calcium salts. The quenching effects of ashed kidney and bone upon the fluorescence of uranium-sodium fluoride fusions were determined and found to be about 20 per cent in the presence of 50 µg of tissue ash.

Uranium Organic Complex:

Because of the importance of the beryllium citrate complex, the polarography of uranyl ion in citrate medium is being reinvestigated. Although beryllium itself does not give polarographic waves, information regarding the physical chemical behavior of beryllium can be obtained by competition studies with uranyl ion.

Problem Code: U.3 (Toxic Limits)

Section Code: 3210

The Relation of Particle Size of Uranium Dioxide Dust to Toxicity Following Inhalation by Animals:

The relation of particle size to toxicity of uranium dioxide dust following inhalation by animals has been determined in 2 parallel studies. In both studies, 16 rabbits and 24 rats were exposed to UO_2 dust at

approximately 80 mg/m^3. In the first experiment, the mass median (M_g) particle size was 0.45 μ. In the second experiment at the same dust concentration, the UO_2 had a mass median particle size above 1 μ. The size fractions were prepared by sedimentation methods. The dusty atmospheres were produced by means of an aspirator feed that developed an aerosol from a water suspension of UO_2. Collection of dust samples by the Cascade Impactor daily permitted a close check on particle-size variation; the Filter Paper Dust Sampler was used to determine concentration. Particles from the "fine" suspension were found by the inert gas adsorption method to have a total surface area ten times that of the particles in the "coarse" suspension, the difference resulting largely from the difference in particle size.

In the rabbit, (a) proteinuria, (b) elevation of urinary amino acid N-creatinine ratio, and (c) elevation of blood NPN occurred in both experiments but was significantly greater at 0.45 μ particle size. Renal pathology in the rabbit was about the same in both studies. In the rat, however, slight but definite renal damage was observed in nearly all animals exposed to UO_2 of 0.45 μ size, whereas practically none was observed in rats exposed to the larger particle sizes. Appreciable lung damage occurred in rats from the 0.45 μ particles; none from the particles of M_g greater than 1 μ. Retention of UO_2 in the lung in both species was 2-5 fold greater at the smaller particle size than at the larger. Table 1 (Page 33) of the original report on the uranium content of the tissues is included because it demonstrates in addition two striking facts about retention of inhaled, insoluble uranium dust: (1) the great difference in the amount of uranium retained in the lung compared with that simultaneously reaching the kidney and bone; and (2) the great differences that result from changes in particle

Table I Uranium Content of Tissues of Animals Exposed for 37 Days to 80 mg U/m³ Uranium Dioxide of Different Particle Sizes

Species	Dust Particle Size	No. Specimens Analyzed	μg U per g ash	
			Mean	Range
	μ	LUNG		
Rabbit	0.45	3	218,000	185,000-244,000
"	1.0	10	37,000	8,000- 48,000
Rat	0.45	5	123,000	35,000-204,000
"	1.0	12	51,000	17,000- 81,000
		KIDNEY		
Rabbit	0.45	7	118	25 - 302
"	1.0	10	256	121 - 610
Rat	0.45	8	304	105 - 842
"	1.0	12	315	10 - 606
		FEMUR		
Rabbit	0.45	8	5.3	1.0 - 17.5
"	1.0	9	11.9	4.4 - 25.2
Rat	0.45	8	10.0	0.8 - 21.6
"	1.0	12	7.6	1.5 - 17.1

size of the inhaled dust. The analyses averaged at the end of 37 days of exposure show approximate ratio of:

40,000 : 25 : 1

for the lung, kidney and femur, respectively for the rabbit inhaling UO_2 dust particles of a mass median size of 0.45 μ. It is to be emphasized that these values were determined on an ash weight basis of both the soft and hard tissues which weights the figures in favor of the soft tissues, the femur containing 60 times the quantity of ash of the lung.

Strikingly different ratios are shown for the same animal species inhaling UO_2 particles of mass median greater than 1 micron. The ratios for the above tissues in order are:

3,000 : 20 : 1

showing, we believe, differences in the manner in which larger particles are handled by body processes. It is immediately apparent from Table 1 that firstly the inhaled quantity of dust is smaller with the larger particle size and such processes as ciliary and phagocytic action is greater, all of which aid elimination of the larger particle size dust from the lung.

In the rat, similar ratios determined for the three tissues for the 0.45 μ particle size are:

12,000 : 30 : 1

for the larger size:

6,000 : 40 : 1

It thus is apparent from these comparisons that the lung serves as a relatively large reservoir for insoluble dust and that within a month only a minute portion gains access to other sites, notably to the kidney and

bone. The approximate constancy of the ratios of kidney to femur uranium concentrations is in sharp contrast to the varying amounts in the lung and shows that the solvation from the lung is sufficiently slow so that the kidney is not overwhelmed by uranium. The processes of elimination maintain approximate equilibrium level of uranium in the blood so that only trivial amounts reach the femur.

Problem Code: U.4 (Fate)

Section Code: 3210

Deposition, Accumulation, and Elimination of Uranium:

Comparison of the amounts of uranium deposited in the tissues of animals exposed to five different compounds for one year, followed by one year of exposure to U-nitrate have revealed for the first time some interesting and useful information on the rate of uptake of uranium in bone and soft tissue, as well as its elimination from tissues.

Animals were exposed to two insoluble uranium dusts, UO_2 and UF_4, and three soluble dusts, U-nitrate, UF_6 and UCl_4.

Groups of approximately 20 dogs and 25 rats were exposed on a daily schedule of 6 hours throughout a $5\frac{1}{2}$-day work week for a period of one year to each of the 5 uranium dusts; in the following year, 3 dogs and 3 or 4 rats from each of these groups were exposed on a similar schedule to a dust of uranium nitrate hexahydrate. During the first year, the concentration levels of exposure were set differently for each of the dusts because of the previously established wide difference in the response of animals to the different compounds; the exposure level of UO_2 was 10 mg. U/m^3, for UF_4

3 mg. U/m^3; for the soluble compounds, U-nitrate was used at 2 levels, 2 and 0.2 mg. U/m^3; UF_6 and UCl_4 were used each at 0.2 mg. U/m^3 for the first year. For the second year, small groups of dogs and rats from each of these levels were all exposed to a single level of 2 mg. U/m^3 of U-nitrate.

The number of animals in each group exposed for the second year from which the data are derived is unfortunately small, firstly, because the experiment was intended to be merely a pilot test for future, more extensive work, and secondly, because death from old age unavoidably removed a significant number of animals from the groups exposed for the second year. This was especially true among the rats which were young adults at the start of the two-year study. Approximately 65 per cent of the rats of the strain used in these experiments normally die within two years under the conditions of diet and housing existing in our colony.

The results of fluorophotometric analysis of animal tissues for uranium content have just come to hand from these experiments that were completed a year ago. The more important conclusions may be summarized briefly thus:

1. Ninety-nine per cent of uranium present in the lungs and 92 per cent of that in pulmonary lymph nodes of the dog at the conclusion of one year of daily inhalation of UO_2 at 10 mg/m^3 is eliminated from these tissues by the end of the second year when exposure is continued to a soluble dust. Eighty-eight per cent is eliminated from the lung of the rat under similar conditions. (See Table 1, Page 37).

2. Elimination from the lung and its lymph nodes of UF_4 (a somewhat less insoluble compound than UO_2) is complete in rats, and probably in dogs, within one year while being exposed to uranium nitrate (Table 1).

3. No overall increase in uranium content occurred in soft

Table 1. Elimination of Uranium From Lungs of Animals After Exposure by Inhalation to the Insoluble Uranium Dusts, UO_2 and UF_4

Soft tissues, wet weight; osseous tissues, ash weight

Tissue	AVERAGE TISSUE URANIUM CONTENT FOLLOWING			Ratio of 1st to 2nd Year's Retention of Uranium	Remarks
	1st Year Exposure	2nd Year U-Nitrate 2 mg U/m3	2 Yr. 1 Mo. Exposure to U-Nitrate 2 mg U/m3		
	µg U/g	µg U/g	µg U/g	%	
	UO_2 10 mg/m3	DOG			After 1 year (during exposure to U-nitrate) an average of 93% of UO_2 is eliminated from lungs and pulmonary lymph nodes of dogs and rats (obtained by subtracting tissue U-content of animals inhaling U-nitrate from that found in animals exposed to UO_2 followed by U-nitrate) The ratio is the relation of this value to the uranium content at the end of the first year.
	N=14	N=1	N=2		
Lung	953	9.8	1.7	0.9	
P.L. Nodes	2514	211	2.9	8.3	
		RAT			
	N=25	N=3(a)	N=3(b)		
Lung	277	35	1.6	12	
	UF_4 3 mg/m3	DOG			The apparently high retentions of UF_4 at the 2nd year are probably the result of small differences between the amounts of U at end of 1st and 2nd years. The possibility of sampling errors here are greater than with UO_2 where the amounts obtained are much larger.
	N=9	N=3	N=2		
Lung	12.3	12.3	1.7	86	
P.L. Nodes	77.6	20.9	2.9	23	
		RAT			
	N=18	N=3	N=3		No retention of UF_4 indicated in rats after 1 year.
Lung	7.3	1.1	1.6	0	

(a) Exposed 11.5 mo. to U-nitrate, 2 mg U/m3
(b) " 6.5 mo. " "
N = Number of animals examined.

tissues of dogs at the end of two years compared with that found at the end of one year when exposure is continued with uranium nitrate at 2 mg. U/m^3. (See Table 2, Page 39). Bone is thus the only site of appreciable accumulation from prolonged exposure to a soluble uranium compound at the relatively high inhalation level of 2 mg. U/m^3.

5. A ten-fold increase in the concentration of a soluble uranium dust during the second year resulted in a two-fold increase in uranium content of the soft tissues of dogs; under the same conditions of exposure, approximately a fifty-fold increase occurred in hard tissue, but the data are scanty. The uranium content of soft tissues of rats increased five-fold from a ten-fold increase in exposure concentration, approximately twenty-five-fold in bone. (See Table 3, Page 40).

6. The uranium content of all tissues of dogs increased from two- to twenty-fold upon a ten-fold increase in exposure dust concentration when the compound inhaled was changed from UF_6 to U-nitrate at the end of the first year. No increase in the lung of the rat resulted from a similar ten-fold increase in exposure concentration, but approximately a five-fold increase was observed in bone. (See Table 4, Page 41).

7. In neither group of dogs exposed to low concentrations of UCl_4 and UF_6 (0.2 mg. U/m^3) for one year and then exposed for another year to U-nitrate (2 mg. U/m^3) was any difference in uranium content of the tissues seen compared with dogs exposed to the same two concentrations of uranium nitrate for two successive years. It may be concluded, therefore, that the past history of exposure to uranium dust (where low concentrations are concerned) has no effect on the deposition of uranium from subsequent exposure at a higher concentration (ten-fold). (Tables 3 and 4).

Table 2. Uranium Deposition after 1 and 2 Years in Animals Exposed by Inhalation to Uranium Nitrate Hexahydrate (2 mg U/m3)

Tissue	AVERAGE TISSUE URANIUM CONTENT FOLLOWING EXPOSURES OF				Remarks
	1 Year N=5	1 Yr. 3 mo. N=3	1 Yr. 6.5 mo. N=3	2 Yrs. 1 mo. N=2	
	µg U/g	µg U/g	µg U/g		
			DOG		
Lung	1.5	1.9	0.4	1.7	No over-all increase in U content in soft tissues of dogs at 2 years over that in 1 year; apparent transient increases only.
P.L. Nodes	1.6	4.6	3.1	2.9	
Kidney	1.7	3.2	0.2	2.0	
Thyroid	1.5	2.6	9.6	0.9	
Adrenal	0.5	0.7	0.7	0.9	
Gonad	0.3	0.3	0.4	0.4	
Liver	0.7	-	4.5	-	
Spleen	0.2	0.1	0.7	0.1	
Pancreas	0.1	0.2	0.2	0.3	
Bone					Content of bone doubled or tripled in second year over that in the first year.
Femur(a)	7.5	26.3	288	12.8	
Rib	-	-	-	17.7	
Skull	-				
Tooth	12.6	30.8	32.7	-	Conclusion: Bone is the only site of apparent accumulation of uranium from prolonged exposure to soluble uranium compounds.

(a) Or elbow
N = Number of animals examined.

Table 3. Uranium Deposition After 1 and 2 Years in Animals Exposed by Inhalation to Soluble Uranium Dusts

Soft tissues, wet weight; osseous tissues, ash weight

Tissue	AVERAGE TISSUE URANIUM CONTENT FOLLOWING EXPOSURES OF: 1st Year UCl_4 0.2 mg U/m³	2nd Year U-Nitrate 2 mg U/m³	1st Yr. U-nitrate, 0.2 mg/m³ 2nd Yr. " 2 mg/m³	Remarks
	µg U/g	µg U/g	µg U/g	
		DOG		U-content of soft tissues of dogs increased 2-fold following 10-fold increase in concentration of soluble U-dust; approximately 50-fold increase in hard tissue, but data are scanty. No difference in U-content of any tissue of dogs first exposed for 1 year to UCl_4 (0.2 mg U/m³), then exposed for another year to U-nitrate (2 mg U/m³) and dogs exposed to the same two concentrations of U-nitrate for two years, i.e. part history of exposure for low concentrations of U dust has no effect on deposition of U from higher concentrations.
	N=14	N=3	N=3	
Lung	1.1	3.0	2.6	
P.L. Nodes	2.6	5.2	5.3	
Kidney	0.2	2.6	19.8	
Thyroid	0.9	1.2	5.3	
Adrenal	1.4	2.2	0.5	
Gonad	0.6	0.4	0.5	
Bone			-	
Femur(a)	0.5	14.3	63.2	
Rib	-	26.6	-	
Skull	-	27.5	-	
Tooth	0.6	-		
		RAT		U-content of soft tissues of rats increased 5-fold from 10-fold increase in concentration; approximately 25-fold in bone.
	N=25	N=4		
Lung	0.6	3.0	-	
Kidney	0.4	-	-	
Femur	0.2	12.4	-	

(a) Or elbow joint
N = Number of animals examined.

Table 4. Uranium Deposition After 1 and 2 Years in Animals Exposed by Inhalation to Soluble Uranium Dusts

Soft tissues, wet weight; osseous tissues, ash weight

Tissue	AVERAGE TISSUE URANIUM CONTENT FOLLOWING EXPOSURES OF: 1st Yr. UF_6 0.2 mg U/m³	2nd Yr. U-nitrate 2 mg U/m³	1st Yr. U-nitrate, 0.2 mg/m³ 2nd Yr. " 2 mg/m³	Remarks
	µg U/g	µg U/g	µg U/g	
		DOG		U-content of all tissues of dogs increased from 2 to 20-fold upon a 10-fold increase in exposure dust concentration. No difference in uranium content of tissues of dogs first exposed for 1 year to UF_6 (0.2 mg/m³) then exposed for another year to U-nitrate (2 mg/m³) and dogs exposed to the same concentrations of U-nitrate for 2 years. Past history of exposure has apparently little effect on accumulation of uranium from higher concentrations
	N=14	N=3	N=3	
Lung	0.9	8.4	2.6	
P.L. Nodes	1.2	25.9	5.3	
Kidney	0.4	3.2	19.8	
Thyroid	0.5	3.0	5.3	
Adrenal	0.3	0.6	0.5	
Gonad	0.4	0.8	0.5	
Bone				
Elbow(b)	5.3(a)	24.3	26.3	
Rib		34.1	-	
Tooth	0.8(a)	-	-	
		RAT		No increase in lung of rat from 10-fold increase in concentration; approximately 5-fold increase in bone.
	N=25	N=1		
Lung	1.2	0.8	-	
Kidney	2.7	-	-	
Femur	2.7	13.7	-	

(a) One high value had distorted the mean

(b) Or elbow joint

N = Number of animals examined.

8. From data not shown in the accompanying tables but appearing in Rochester Report No. UR-30, another important conclusion was reached regarding the retention of the uranium in the body from inhaled soluble uranium dust. The accumulation of uranium in the bone of animals breathing uranium nitrate becomes appreciable only at concentrations greater than 0.25 mg. U/m^3. At lower concentrations elimination appears to keep pace with uptake. Even at the higher concentrations (2 mg. U/m^3) and after prolonged exposure (one year), however, very little uranium remains in the soft tissue; bone is the chief site of deposition of a soluble uranium dust.

Further information that is required but is not yet available is that of the elimination of uranium from bone. Experiments are planned to yield this type of information.

Ca^{45} Adsorption:

Previous studies on the exchange of the calcium of bone with the calcium of radioactive $CaCl_2$ solutions of different concentrations have been continued. They include the comparison of the exchange of calcium on fresh bone and on 80 - 100 mesh glycol-ashed bone. The previous work has been expanded to include a study of the adsorption of Ca^{45} on bone ash from 10^{-4} M $CaCl_2$ solution during exposures ranging from $\frac{1}{2}$ hour to 14 days. Likewise, bone ash previously exposed to radioactive 10^{-4} M $CaCl_2$ solution is being shaken with nonradioactive $CaCl_2$ solution of the same concentration for periods of from $\frac{1}{2}$ hour to 14 days; in these experiments the initial exposure of the bone was always 14 days. The purpose of this experiment is to study the desorption of Ca^{45} from bone into solution. In all cases the radioactivity and the Ca concentration is determined in the bone and the solutions.

The results of these and previous studies at higher Ca concentrations (10^{-3} M and 10^{-2} M) are being compiled and evaluated.

Rickets: The studies on the mechanism of action of cod liver oil on the healing of rickets in rats with Ca^{45} as indicator of the calcium metabolism of bone continues.

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

Section Code: 3220, 3260

Mechanism of Action of Uranium on Isolated Cells:

It has been shown previously that uranium acts on the surface of the yeast cell (Rochester Report No. UR-8) and that a complex is formed between uranium and certain groups on the cell surface (Rochester Report No. UR-17). The uranium complexing groups of the cell are involved in the metabolism of glucose, presumably in the phosphorylation of glucose to glucose-6-phosphate by the hexokinase-adenosine triphosphate (ATP) system.

The experiments reported here suggest that uranium inhibits glucose metabolism by complexing with the polyphosphate chain of ATP bound on the cell surface. The uranium probably replaces the Mg ion which is the activator of the ATP hexokinase system.

A comparison was made between the dissociation constants of the uranium complex with yeast surface groups and that of a number of pure compounds. The complexes with polyphosphates such as inorganic pyro- and tri-phosphate and ATP had dissociation constants of the same order of magnitude as that with yeast. However, the complexes with ortho-phosphate, glucose-6-phosphate, glycerophosphate, citrate and protein (calculated on the basis of

carboxyl groups) were considerably lower.

In preliminary experiments, it was shown that Mg^{++} and Ca^{++} compete with UO_2^{++} for the yeast cell surface groups. However, the uranium complex is much tighter than that with magnesium or calcium.

PROGRAM Be.

BERYLLIUM

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

Section Code: 3210, 3220

Particle Surface Area Determinations of Various Grades of Beryllium Oxide:

Because a correlation was believed to have been found between the incidence of toxicity and exposure to different grades of beryllium oxide among industrial workers, a reasonable experimental approach to the understanding of this problem appeared to be the determination of certain physical properties of the various oxides. Most critical of these as regards toxicity is that of surface area. Accordingly, the surface area of seven different grades of Be oxide bulk samples have been determined by the method of low temperature adsorption of ethane which has been described briefly previously in a technical report.

The surface area and particle size values of the different grades of Be oxide are shown in Table 1 (Page 46). Two of the samples, the GC and the SP grades of Company "P" showed relatively high particle size, 40 and 20 μ respectively. The size mass medians of the remaining samples, however, were all within the relatively low range from 2 to 3 μ. The determination of these particle sizes was made by optical measurement of the particles and in the case of the fluorescent grade oxide, sizes were assigned to the clumps as it was not possible by the optical method to measure accurately further subdivisions of these clumps. This accounts for the similarity in the mass median of the refractory and fluorescent grades of the oxides which appear widely different in electron microscope pictures.

Table 1. Surface Area Determinations by Low Temperature Ethane Adsorption on Beryllium Oxides

Origin of Sample	Sample No.	Sample Description	Firing Temp. °C	Particle Size Mass Median μ	Surface Area by Ethane Ads. m²/g
Co. "P"	BB-18	Lot #901	1350	2.41	2.72
	BB-25	Lot #1789 GO Grade	1150	39.6	0.39
	BB-26	Lot #939 SP Grade	1400	20.5	2.42
Co. "R"	BB-16	Grade B	2580	2.93	0.66
	BB-17	Grade A	1150	2.99	14.7
Co. "Q"	BB-19	--	400	3.00	53.5
	BB-20	--	1000	2.13	1.28

The highest values of surface area were assigned to the oxide fired at 400°C from Company "Q" with a value of 53.5 m^2/g; next in order was Grade "A" of Company "R" with a surface area of 14.7. The lowest in area was Grade "B" of this latter company and the GC grade from Company "P". In general, it is seen that there is an approximate surface area relation between the fired temperature of the oxide and the specific surface, namely, the higher the area the lower the firing temperature.

It will be a matter of considerable interest to correlate from results of toxicity studies now underway what, if any, relations exist between these physical measurements and the effects in animals.

Problem Code: Be.3 (Toxic Limits)

Section Code: 3210, 3220, 3230

Concentration Toxicity, Intravenous Injection:

Using several different methods, three types of beryllium hydroxides have been prepared and the intravenous toxicity of each determined in rats. "Beta" beryllium hydroxide, gave an LD50 of 2.5 mg. of Be/kg. "Alpha" beryllium hydroxide about 0.8 and "Alpha" beryllium hydroxide containing small quantities of citrate gave an LD50 of approximately 0.35 mg/kg.

Pilot Beryllium Oxide (Grade "A") Inhalation Study:

The toxicologic effect of beryllium oxide dust, Company "R", (Grade "A"), was investigated in a preliminary inhalation exposure study in animals.

Following three weeks of conditioning in a beryllium free exposure chamber, 20 rats, 10 guinea pigs, and 3 rabbits were exposed to a mean concentration of 87 mg. $BeO/m^3 \pm 10.5$ mg. BeO/m^3 for a total of 60 hours over a

twelve-day period. At the conclusion of exposure, all animals except 5 rats, which were saved for further observation, were sacrificed and tissues submitted for histologic study.

Only 2 animals, rats, died during the experiment. One rat died on the 3rd day of exposure; the other, one week following the termination of exposure.

No loss in body weight was observed in any species during exposure; however, 2 of the 5 rats not sacrificed showed post-exposure weight loss.

Urinary protein and blood nonprotein nitrogen in the rabbits were consistently normal. Serum protein and albumin/globulin ratio done weekly on the same animals also showed no significant changes.

Hematologic studies on the rabbits gave no indication of changes in blood picture. Hemograms on the rats, however, showed changes that warrant further investigation. Three of the 5 rats that were held for further observation had a slight leukocytosis during the exposure period. This increase became more marked during post-exposure (See Table 1, Page 49). The rise in total white blood cell count was accompanied by an increase in both neutrophils and lymphocytes.

The histologic study of tissues of the sacrificed animals has not been completed.

It is to be concluded that a two-week exposure to BeO, "A" grade, at 87 mg/m^3 gives indications of potential toxicity to laboratory animals.

Acute, Massive Exposures to Beryllium Oxide and Hydroxide:

A study has been completed for the purpose of determining whether exposure to various grades of oxide of beryllium can cause an acute type of

Table 1. Mean White Blood Cell, Neutrophil and Lymphocyte Counts in Rats Exposed to 85 mg/m³ BeO Dust, Clifton Fluorescent Grade. Company "R" (Grade "A")

Period		Pre-Exposure	Exposure	Post Exposure
Number of Weekly Counts		3	2	2
Mean White Blood Cell Count	Rat #6	10,833	13,800	17,500
	#8	16,566	21,150	34,500
	#9	14,250	18,420	29,850
	#10	22,166	21,225	26,975
Mean Absolute Neutrophil count	Rat #6	3,192	5,616	6,451
	#8	8,653	7,498	20,056
	#9	4,073	7,026	9,857
	#10	11,881	9,816	12,110
Mean Absolute Lymphocyte Count	Rat #6	7,300	7,552	10,413
	#8	6,979	13,440	14,054
	#9	9,372	11,215	18,798
	#10	9,210	11,081	14,246

reaction in the lung, and especially whether such a reaction can result from a single massive exposure to beryllium oxide dusts.

The experiments relating to this study fall into three general groups. In the first, small groups of 5 to 10 rats each were placed in a small exposure chamber measuring 18" on a side, and exposed to a high concentration of beryllium oxide dusts at levels of the order of 1500 milligrams per cubic meter. Various grades of beryllium oxide were used, and the conditions of exposure were maintained in each case for a period of one hour. In most cases the animals were sacrificed 12 days after a single exposure of this type, and autopsy data recorded although the two compounds which appeared to be most active after a single exposure were retested in a series of repeat exposures, one hour each day for twelve days.

The second group of experiments was conducted in the same way insofar as exposure to beryllium was concerned, but in addition, certain physiological stresses were superimposed such as enforced exercise, heat and cold.

In the third group of experiments, each of the various compounds was injected intratracheally into groups of 5 to 10 rats at a uniform dosage level of 100 milligrams per kilogram of body weight. Each group was sacrificed after 12 days.

During the 12 days of each experiment, each animal was examined daily and the following data recorded: body weight; intensity of respiratory noises (râles); presence or absence of sniffles, exudate from the eyes and infections of the middle ear, scoring each attribute on an arbitrary scale of from 1 to 10 units. On the 12th day, each animal was sacrificed and gross pathological changes recorded, including a numerical estimate of the degree of lung damage (scale 0 to 10) and the relative weight of each lung

expressed as a percentage of the body weight as indicated in Table 2 (Page 52).

Results: Only a few of the data recorded showed any evidence whatsoever of a consistent trend related to the details of the exposure. Thus, changes in body weight ranged from a mean weight loss of 0.03 grams per day per rat to a mean weight gain of 3.8 grams per day per animal, with almost no correlation ($\sigma = 0.03$) with findings at autopsy, largely because of the high curvature of the normal growth curve for rats in the age group used. Symptoms such as presence of sniffles and bloody eyes appear too sporadically to permit adequate evaluation in such limited groups as were used. Respiratory noises in rats exhibit a complex picture which is difficult to interpret; as a result, little correlation can be demonstrated between rales and autopsy findings.

The autopsy data are by far the most consistent of the findings. Individual animals within each group are mutually consistent, and in addition, the recorded values for relative lung weight and apparent lung damage show a high degree of correlation ($\sigma = 0.6$). For this purpose, only the autopsy findings are shown in Table 2.

Conclusions: All oxygen compounds of beryllium studied cause definite changes in the lungs of white rats whether administered by inhalation or by intratracheal injection. None of these changes appear to impair the general well-being of the animal, or at least the impairment is not so great that it is readily demonstrable by any external tests yet devised, but is evident only in autopsy material. It is not yet possible to state positively whether this failure to show external changes is due to the absence of such changes, or to the lack of sensitivity in the tests employed or more probably

Table 2. Acute (Short-Term) Response to Various Grades of Beryllium Oxide and Hydroxide

A. Sacrifices made twelve days after a one-hour exposure

Compound Tested	Apparent Gross Lung Damage	$\frac{\text{Lung Wt}}{\text{Body Wt}} \times 100$
Co."P" Oxide, SP Grade	3.2	0.45
Co."R" Oxide, Grade A	3.2	0.46
Co."R" Hydroxide	3.6	0.42
Oxide freshly ignited from beryllium hydroxide	2.8	0.87
Co."R" Grade A after 12 daily 1-hour exposures	2.2	0.94
Same, 12 days later	2.8	0.95
Co."R" Hydroxide after 12 daily 1-hour exposures	3.4	1.25
Same, 12 days later	4.6	1.60

B. Sacrifices made twelve days after a one-hour exposure to beryllium oxide with additional physiological stress

Compound	Stress	Gross Lung Damage	$\frac{\text{Lung Wt}}{\text{Body Wt}} \times 100$
Co."R" Oxide	Heat during exposure	6.1	1.3
Co."R" Hydroxide	" " "	2.4	0.97
Co."R" Hydroxide	Heat after exposure	1.4	0.64
Co."R" Hydroxide	Cold after exposure	1.6	0.70
Co."R" Hydroxide	Exercise after exposure	2.4	0.75

C. Sacrifices made twelve days after intratracheal injection of various compounds at 100 mg/kg

Compound	Gross Lung Damage	$\frac{\text{Lung Wt}}{\text{Body Wt}} \times 100$
No injection	2.2	0.66
Saline vehicle only	2.2	0.65
Co."P" oxide, SP grade	4.0	0.88
Co."R" oxide, Grade A	4.0	0.89
Co. "R" hydroxide	3.5	0.90
Co."Q" 400° oxide	4.8	1.19

to the short duration of the experiment. The failure of any of the treatments (with one exception) to cause the death of any animal within the time limits described would seem to imply that the changes produced are actually of very moderate degree.

The data obtained from the animals which were intratracheally injected indicate that beryllium hydroxide is at least as active as the oxide which is prepared from it by ignition, while the inhalation data suggest that it is considerably more active. The discrepancy is undoubtedly due to the fact that the hydroxide has some peculiar physical properties which make it very liable to suspension in the air in stable aerosols of a smoke-like character. The oxide prepared by Company "Q", on the other hand, appears to be toxicologically more active than any of the other materials studied.

The data further indicate that exposure to beryllium at elevated temperatures (100-105°F) is more damaging than at normal temperatures. It is highly significant that at only these high temperatures were any animals actually killed by the treatment.

Repeated exposures up to 12 days' duration produce changes comparable with the increased exposure. On the other hand, the data for the hydroxide suggest that the changes produced may be of a progressive character, increasing in intensity after the physical exposure is terminated.

Summary: Exposure to the insoluble oxygen compounds of beryllium are shown to produce measurable changes in the lungs of rats after one or several exposures. It is not possible on the basis of the present data to state whether these changes represent a serious threat to the survival of the animals, especially since there is some indication that the lesions may be active and progressing 12 days after the exposure to beryllium has been terminated.

Acute Toxicity of Beryllium by Intraperitoneal Route:

Cumulative mortality of rats following intraperitoneal injections with various doses of Be oxide (Grade "A" and Grade "B") has been determined. Male rats, average age 55 days, were injected intraperitoneally with single doses of a suspension containing 50 mg/cc. The results are shown in Tables 3 and 4 below.

Table 3. Be Oxide (Grade "A")

	WEEKS								
Dose (mg/kg)	1	2	3	4	5	6	7	8	9
3000 (Saline)	0/10	3/10	4/10			4/10			
3000 (Aqueous)	0/10	2/10		4/10			5/10		
2000 (Saline)	1/10					1/10			
2000 (Aqueous)	2/10	3/10	4/10				4/10		
2000 (Saline)*	6/10	7/10	8/10					8/10	
1000 (Squeous)	0/10		1/10						1/10
1000 (Saline)	0/10	1/10							1/10

*Suspension prepared 4 months previous to injection.

Table 4. Be Oxide (Grade "B")

	WEEKS								
Dose (mg/kg)	1	2	3	4	5	6	7	8	9
2000 (Aqueous)	0/10			1/10			2/10	3/10	3/10
1000 (Aqueous)	0/10	1/10					2/10		2/10
1000 (Saline)	0/10								0/10

Numerator indicates number of animals that died; denominator the number of animals injected.

It is apparent that the acute toxicity from the two grades of BeO is very low. Grade "B" appears to be less toxic than Grade "A" by the intraperitoneal route.

Acute Toxicity by Ingestion:

Data from pilot experiment in which groups of 10 ♂ and 10 ♀ rats (4 weeks old) were fed various dietary levels of Be carbonate ($BeCO_3$. 2BeOH.$3H_2O$) for 6 weeks. The results are shown in Table 5 below.

Table 5.

Diet %	Mortality ♂	Mortality ♀	Initial Bd. Wt. Average (g) ♂	Initial Bd. Wt. Average (g) ♀	Final Bd. Wt. Average (g) ♂	Final Bd. Wt. Average (g) ♀	Bd. Wt. Change Average (g) ♂	Bd. Wt. Change Average (g) ♀
Controls	0/10	0/10	51	49	208	146	+157	+97
0.5%	0/10	0/10	52	51	208	147	+156	+96
2.5%	0/10	0/10	52	52	189	140	+137	+88
5.0%	1/10	2/10	53	51	107	91	+ 54	+40

Radiographic Findings in Tibial Head:

Controls, negative; 0.5 per cent, negative; 2.5 per cent, bone lesion (slight); 5.0 per cent, pronounced "rickets-like" bone lesion.

Basic beryllium carbonate is demonstrably toxic to rats at a dietary level of 2.5 per cent and produces death in some rats at double this level.

Chronic Toxicity by Ingestion:

Data from experiments in which groups of 25 ♂ and 25 ♀ weanling rats were fed Be sulfate (tetrahydrate) at a dietary level of 5 per cent are given in Table 6 below.

Table 6.

First 31 Days of Experimental Period

		Mortality ♂	Mortality ♀	Initial Bd. Wt. Average (g) ♂	Initial Bd. Wt. Average (g) ♀	Final Bd. Wt. Average (g) ♂	Final Bd. Wt. Average (g) ♀	Bd. Wt. Change Average (g) ♂	Bd. Wt. Change Average (g) ♀
Controls	Stock diet	0/25	0/25	49	45	177	130	+128	+85
Experimentals	5% Be sulf.	2/25	2/25	48	32	55	51	+ 7	+ 9

Radiographic Findings in Tibial Head:

Controls, negative; experimentals, pronounced "rickets-like" condition.

31st Day to 42nd Day of Experimental Period

Diet		Mortality	Bd. Wt. 31st Day Average (g) ♂	Bd. Wt. 31st Day Average (g) ♀	Bd. Wt. 42nd Day Average (g) ♂	Bd. Wt. 42nd Day Average (g) ♀	Bd. Wt. Change Average (g) ♂	Bd. Wt. Change Average (g) ♀
Controls	Stock Diet	0	177	130	217	149	+40	+19
Experimentals	Stock Diet	0	55	51	112	91	+57	+40

Radiographic Findings in Tibial Head:

Controls, negative; experiments, "rickets-like" condition partly "healed".

42nd Day to 49th Day of Experimental Period

Diet		Mortality	Bd. Wt. 42nd Day Average (g) ♂	Bd. Wt. 42nd Day Average (g) ♀	Bd. Wt. 49th Day Average (g) ♂	Bd. Wt. 49th Day Average (g) ♀	Bd. Wt. Change Average (g) ♂	Bd. Wt. Change Average (g) ♀
Controls	Stock Diet	0	217	149	232	155	+15	+6
Experimentals	Stock Diet	0	112	91	139	109	+27	+18

Radiographic Findings in Tibial Head:

Controls, negative; experimentals, healing of "rickets-like" condition-now healed; new radiolucent area now present in proximal portion of tibial head.

A hitherto unreported finding of changes progressing in the tibial head of the femur of rats from the ingestion of soluble beryllium sulfate has been demonstrated. The newly discovered change occurs after the apparent healing of the rickets-like condition on the 5 per cent diet and is

demonstrable by x-ray approximately one month after the establishment of the ricket condition.

Effect of Injected Beryllium on Number of Offspring in Rats:

Appended are data for rats from a breeding experiment in which the females as weanlings were injected intraperitoneally with a single 1000 mg/kg dose of Be oxide. None of the male rats in this experiment were injected with Be oxide. The rats were paired (one pair per cage) at weaning; 25 pairs were used in each group.

Table 7.

Groups - ♀	Mortality - ♀
Controls - Not injected.	0/25
Exp. I - Be oxide (Grade "B") - caged with males	0/25
Exp. II - Be oxide (Grade "A")	4/25
Exp. III - Be oxide (Grade "A") - caged without males	3/25

Percentage of Females Bearing Litters in First Five Months

Litter No.	I	II	III	IV	V	VI
Controls	100%	100%	95%	55%	19%	5%
Exp. I	96%	78%	64%	36%	5%	0
Exp. II	96%	87%	68%	55%	10%	0

The data show that beryllium oxide mothers treated prior to mating with nontreated males have substantially reduced numbers of litters during the five-month period of observation in comparison to untreated controls. The experiment as yet does not permit a conclusion as to the effect of beryllium on reproduction throughout the life span of the rat. The apparent reduction of litters may represent a retardation in the rate of litter production.

Pregnancy in beryllium treated female rats has not changed the rate of mortality in the five-month period of the experiment.

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

Section Code: 3210, 3220, 3260

Change in Blood Lipid Ratios as Index of Beryllium Poisoning:

The differential analysis of blood lipids of animals was undertaken in the hope of finding a clinical method to aid in the diagnosis of beryllium poisoning. It was felt that the important field of lipid chemistry should not be neglected merely because of the difficulty of lipid micromethods. A complete analysis, such as performed in this study, permits no important constituent to escape examination. Accordingly, the partition of lipids in both plasma and red cells of rabbits was determined according to the following scheme of separation:

I Total Lipid

II Neutral Fat

III Total Fatty Acids

- A Phospholipid Fatty Acids
- B Cholesterol Ester Fatty Acids
- C Neutral Fat Fatty Acids

IV Total Cholesterol

- A Ester Cholesterol
- B Free Cholesterol

V Phospholipid

It was found in the normal animal group that concentrations of individual lipids as well as the ratios of one to another were more constant in the red cells than in the plasma. Following the intravenous injection of

the rabbits with 2 mg. $BeSO_4 \cdot 4H_2O$/kg., alterations in the lipid pattern were also more consistent in the red cells. Furthermore, it was found that the ratio of phospholipid to free cholesterol changed more uniformly than any one individual lipid concentration (See Table 1, Page 60). Consequently, this ratio was chosen for further investigation in the red cells of dogs.

In 5 dogs, the normal phospholipid to free cholesterol ratio varied only from 2.4 to 2.6. During daily inhalation exposure to 30 mg. $BeSO_4 \cdot 4H_2O/m^3$, however, this ratio showed a consistent downward trend in 4 of 5 animals (See Table 2 and Figure 1, Pages 60 and 61). At the end of the experiment, values were still decreasing, probably indicating that the experiment was not of sufficient duration or that a chronic phase was setting in. A drop of 0.2 units during a period of a week appears to indicate an unfavorable prognosis. The reversal of the usual downward trend by dog #1225 may perhaps be explained by the fact that only minimal tissue damage was observable by either biochemical study or gross pathology.

The reason for a decline in phospholipid to free cholesterol ratio in dog red cells but an increase in this value in rabbits is not clear.

Effect of Superimposed Beryllium Exposure on Tuberculosis in Guinea Pigs:

In 1935 Loomis and Bogen (1) reported an enhancing effect of various soluble beryllium compounds on the extent of tuberculous infection in guinea pigs and further suggested the treatment of guinea pigs with beryllium to increase the sensitivity of this animal in testing tuberculous sputum. Because of this report and the present industrial interest in the toxicity of beryllium, it was thought advantageous to confirm this observation.

(1) Loomis, R. N. and Bogen, E. The Biological Effects of Beryllium. Am. Rev. Tuberculosis, 32:475-480 (1935).

Table 1. Rabbits Injected Intravenously with 2 mg $BeSO_4 \cdot 4H_2O$/kg

Phospholipid to Free Cholesterol Ratio in the Red Blood Cell

Animal Number	Phospholipid/Free Cholesterol	
	Control	4 Days after Injection
641	2.5	4.2
965	2.4	3.5
967	2.8	3.7
968	2.7	3.6
956	-	3.2
825	-	3.0

Table 2. Dogs Exposed by Inhalation to 30 mg/m^3 Level of $BeSO_4 \cdot 4H_2O$

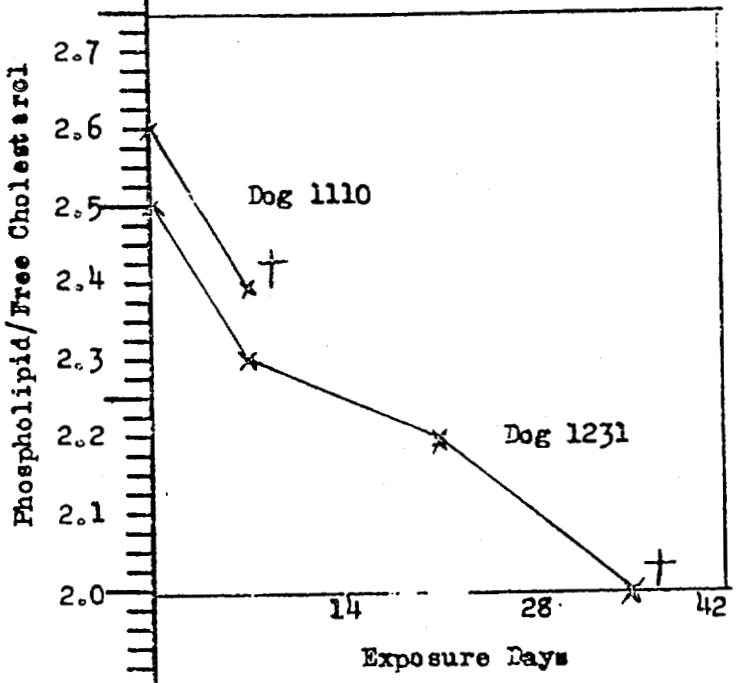
Phospholipid to Free Cholesterol Ratio in the Red Blood Cell

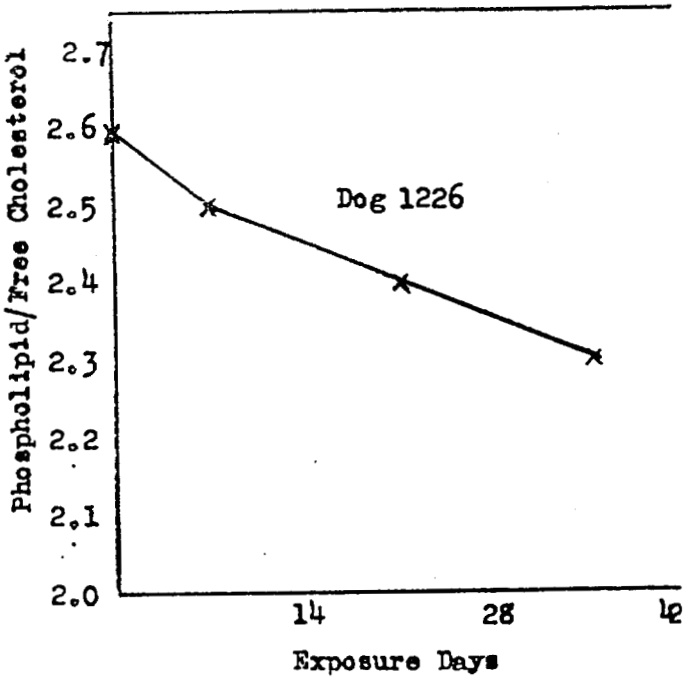
Animal Number	Control	7 Days	21 Days	35 Days
1110	2.6	2.4*		
1226	2.6	2.5	2.4	2.3
1230	2.4	2.4	2.3	2.2
1231	2.5	2.3	2.2	2.0*
1225	2.4	2.4	2.5	2.8

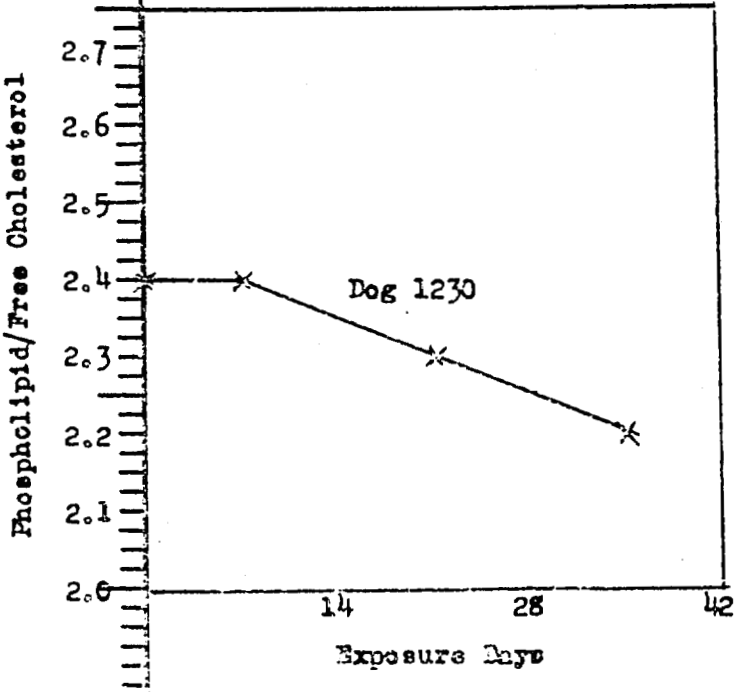
* died

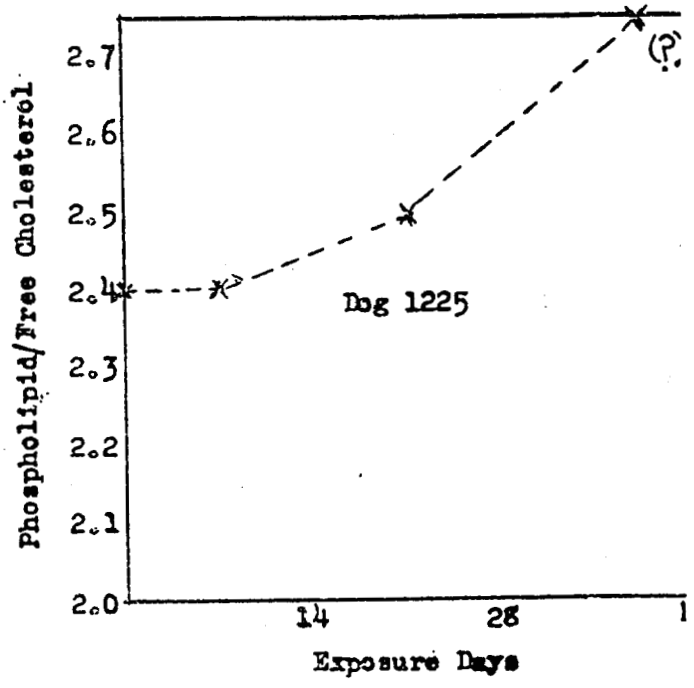
Figure 1. Dogs Exposed by Inhalation to 30 mg $BeSO_4 \cdot 4H_2O/m^3$

Phospholipid to Free Cholesterol Ratio in the Red Blood Cell









Equal numbers of male and female guinea pigs were selected and distributed among groups of 10 each. One group was infected with 0.005 mg. of M. tuberculosis, strain H37 (ATCC 8236). These animals served as control infected animals. Two other groups of guinea pigs received the tuberculous infection in doses of 0.5 and 1 mg. subcutaneously and intra-abdominally, respectively, and in addition received weekly exposure to beryllium sulfate at a concentration of 50 mg/m^3 for from 2 to 6 hours daily. A 4th group received no infection with tuberculosis but was exposed to beryllium sulfate only. A 5th group received neither infection nor exposure to beryllium sulfate and served as normal weight controls for the entire group. Exposure to beryllium was continued for $2\frac{1}{2}$ months. Weights were taken weekly and observations were continued for a period of 4 months from the start of the infection. At this time autopsies were performed on the animals and the results of previously obtained nodular size correlated with the histologic results from stained sections.

The microscopic examination of the tissues of the guinea pigs failed to show that weekly exposures to 50 mg/m^3 of beryllium sulfate had any effect on the course of tuberculosis in the guinea pig. The lymph nodes draining the area of infection showed in all groups the same type of tissue reaction, namely proliferative tubercles some of which were caseous. The liver and spleen of a few animals in each of the 3 infected groups contained a few tubercles of miliary size and of proliferative type but no significant differences between the groups, although the dosage of tubercle bacilli among the groups differed widely.

No pulmonary tuberculosis was found and the pulmonary lesions usually observed from the inhalation of beryllium sulfate were either minimal or

absent; this finding has been previously observed in the guinea pig which is a relatively resistant animal to inhalation of beryllium dust. Accordingly, it may be concluded conversely that the tubercular infection did not predispose guinea pigs to beryllium poisoning.

The findings at gross autopsy and measurement of nodular size, made throughout the study, showed no differences attributable to the different treatments among the groups. Moreover, the weight response of all treated groups showed a parallel rise in growth following the third week of the study which was at all times somewhat less than that of the untreated controls. The beryllium-exposed animals showed the poorest growth response of all groups. In general, however, the weight response was consistent with the histologic findings.

These results with experimental animals agree with the general impression of industrial physicians that beryllium workers with tuberculosis are not more susceptible to beryllium poisoning.

Effect of Beryllium on Isolated Cells:

Beryllium, except in high concentrations (0.01 M) had no effect on the rate of growth and cell division of either Baker's yeast cells or E.coli in a synthetic medium.

Mechanism of the Deposition of Beryllium in Bone:

Because distribution studies have shown beryllium to be deposited in bone, a few preliminary experiments have been conducted to outline the mechanism of its deposition. These indicate that the solubility of beryllium in physiological solutions is so low that chemical methods for the determination of beryllium will not avail in the solution of the problem and that isotopic techniques will be required.

PROGRAM Th.

THORIUM

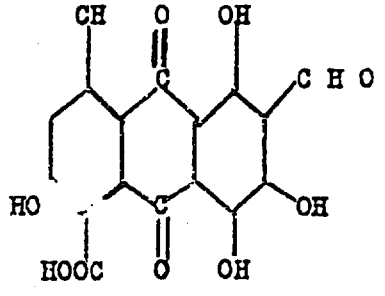
Problem Code: Th.1 (Physical and Chemical Properties)

Section Code: 3210

Quantitative Microanalytic Method for Thorium:

A rapid colorimetric method has been developed for the determination of thorium in microquantities. In acid solution thorium ions form a colored complex with carminic acid, the coloring matter of cochineal. The intensity of color in the solution depends upon the concentration of thorium and of the reagent. The method is an extension of an observation by Beck (Mikrochemie, 27:47 (1939)) who reported that thorium could be detected through the production of a blue color with tincture of cochineal in nearly neutral solution.

Carminic acid is a complex polyhydroxy anthraquinone derivative for which Dimroth has proposed the structure:



(1,3,4,6-tetrahydroxy-2-glucosidyl-8-methyl-anthraquinone-5-carboxylic acid).

In the complex formed with thorium ion, the coordination number is 4, the same as the valence of thorium. The dye is an indicator over the range of pH 4.7 to 6.2 with pK' at about pH 5.5, the color changing from orange through red and magenta to purple. In solutions in which the concentration

of the thorium-carminic acid complex is constant, the intensity of color is a function of the pH. The pK' is shifted toward the acid range from that of carminic acid alone. The optimum pH for application to the determination of thorium is about pH 4.4, and is determined largely by the desirability of keeping the blank correction low. In the range of concentrations of thorium ion between 0 and 20 µg/ml., at constant pH, the relation between extinction and thorium ion concentration follows the Beer-Lambert Law.

Spectral absorption curves were measured with a Beckman Model "DU" quartz spectrophotometer, using the "blue sensitive" photocell and 1 cm. Corex cuvettes. The greatest difference in absorption between the carminic acid solution and that containing thorium ion occurred at about 580 mµ. Using the Beckman quartz spectrophotometer, the maximum sensitivity of the method is one part of thorium in 500,000. The molecular extinction, ϵ, is 4,496 ± 38. With the Klett-Summerson photoelectric colorimeter, using a Farrand interference filter with maximal transmission at 575 mµ, the maximal sensitivity is one part in 400,000. The change in optical density for unit change in Th^{++++} concentration (1 µg/ml) is about 31 scale units.

In the procedure finally adopted, 1 ml. of a 0.14 per cent aqueous solution of carminic acid (about 2.84×10^{-3} M solution) is used in a total volume of 10 ml. of a solution buffered at pH 4.4 with M/2 $KHC_8H_4O_4$ -NaOH or M/2 HOOC·CH3-NaOOC·CH3 sodium acetate and containing 25 to 200 µg total thorium ion. An advantage of the method is that the colored complex between thorium and carminic acid forms almost instantaneously, so that the necessity for heating in order to accelerate development of color is eliminated.

The common anions which form water-soluble salts with thorium ion do not interfere in the determination. The method cannot be used, however, in the presence of anions which precipitate thorium as water-insoluble salts, e.g., fluoride, carbonate, hydroxyl, phosphates, tungstate, molybdate, iodate, and most organic acids except acetate and phthalate. In addition, the method cannot be used in the presence of certain organic anions, e.g., citrate and tartrate, which complex with thorium, and in which the strength of the coordinate bond is greater than that between thorium and carminic acid. Ferrous and ferric iron and aluminum interfere seriously in the determination of thorium. Using the Klett-Summerson photoelectric colorimeter, the sensitivity of the method for ferric iron and for aluminum is about one part in 750,000, while the sensitivity for ferrous iron is about one part in 400,000. Tungsten, molybdenum, uranium, and zirconium form colored complexes with carminic acid, but the sensitivity of the method for the detection of these cations is low compared with that for the determination of thorium.

Problem Code: Th.X (Toxic Effects)

Section Code: 3210

Acute Toxicity of Inhaled Thorium Nitrate Dust:

A pilot experiment was performed in which 58 laboratory animals, comprising four different species, were exposed to thorium nitrate dust in a chamber 6 hours daily for a total of 66 hours during 16 calendar days. The distribution of the laboratory animals among the 4 species was as follows: 25 female mice, 20 rats, equally divided with respect to sex, 10 male guinea

pigs, and 3 rabbits. The purpose of the experiment was to determine whether

[redacted]

is due to the hygroscopicity of the thorium nitrate dust and a certain amount of unavoidable nuisance dust.

No deaths occurred during the exposure period and all of the animals were sacrificed terminally for pathological examination. Gross examination at autopsy revealed moderate pulmonary damage of hemorrhagic nature in a few of the animals, but in most of them, the lungs appeared to be normal. The weight curves showed no significant change in trend during the period of exposure from that during the conditioning period. The biochemical data also failed to reveal evidence of toxic effects. Preliminary analysis of the hematologic findings indicated that there was some disturbance of hematopoiesis in a few of the animals, although in the majority the findings were negative.

PROGRAM F.

FLUORIDE

Problem Code: F.3 (Toxic Limits)

Section Code: 3210, 3230

Acute and Pilot Feeding Studies:

The feeding of sodium fluoride to rats at dietary levels of 0.02 and 0.2 per cent was continued during the past quarter until the total elapsed time on the diets reached six months. At this point all rats were sacrificed with the exception of those on the control diet and those on the 0.2 per cent level, and specimens of tooth, root, crown and incisor, and epiphysis, diaphysis and alveolar bone removed for fluoride analysis. The control rats and those animals on the 0.2 per cent level will be given a 21-day LD50 dose of uranium nitrate to determine whether or not the increased fluoride intake has increased the resistance of the rat to uranium toxicity. (For further discussion of this issue see Problem Code F.4, Page 74).

The urinary fluoride excretion data for the six-month period indicate that immediately after having been placed on the fluoride diets, the level of fluoride in the urine increases steadily until a plateau is reached. An interval of 23 days is required for those animals on the 0.02 per cent diet to reach a fairly constant output. The mean of the plateau at this level of intake is approximately 0.5 mg. F/total volume of urine excreted. A 48-day period is required for those animals receiving 0.2 per cent sodium fluoride to show a plateau in urinary output; the mean excretion for this plateau is approximately 4.5 mg. F/total volume of urine excreted. It will

be noted that this is a nine-fold increase in the urinary level of fluoride; the dietary level is ten-fold increase. A complete report on these feeding experiments will be prepared upon completion of the bone analyses and uranium toxicity studies.

Toxicity of Sodium Fluoride by Ingestion:

Data are presented from an experiment in which groups of male and female rats (5 or 6 of each sex per group) were fed various dietary levels of NaF for a period of 6 months.

Table 1.

First 3 Weeks of Experimental Period

Group	Diet	Mortality ♂	Mortality ♀	Initial Bd. Wt. Average (g) ♂	Initial Bd. Wt. Average (g) ♀	Final Bd. Wt. Average (g) ♂	Final Bd. Wt. Average (g) ♀	Bd. Wt. Change Average (g) ♂	Bd. Wt. Change Average (g) ♀
I	Stock Diet	0/6	0/6	59	57	154	130	95	73
II	0.02% NaF	0/6	0/6	61	56	163	144	+102	88
III	0.2 % NaF	0/6	1/6	64	63	90	78	+ 32	23
IV	0.4 % NaF	5/5	5/5	58	55				
V	1.0 % NaF	6/6	6/6	58	56				

From 3rd Week to End of 6th Month of Experimental Period

Group	Diet	Mortality ♂	Mortality ♀	Initial Bd. Wt. Average (g) ♂	Initial Bd. Wt. Average (g) ♀	Final Bd. Wt. Average (g) ♂	Final Bd. Wt. Average (g) ♀	Bd. Wt. Change Average (g) ♂	Bd. Wt. Change Average (g) ♀
I	0.02% NaF	0		154	130	296	211	+142	+81
II	Stock Diet	0		163	144	290	211	+127	+67
III	0.2 % NaF	0		90	78	232	144	+147	+66
IV	(all dead)								
V	(all dead)								
VI	(new control group)	0		161	144	293	220	+132	+76

Data are presented from an experiment which is still in progress in which groups of 5 male and 5 female mature rats (6 months old) have been fed various dietary levels of NaF for a period of three weeks.

Table 2.

Diet %	Mortality ♂	Mortality ♀	Initial Bd. Wt. Average (g) ♂	Initial Bd. Wt. Average (g) ♀	Bd. Wt. (3 wks) Average (g) ♂	Bd. Wt. (3 wks) Average (g) ♀	Bd. Wt. Change Average (g) ♂	Bd. Wt. Change Average (g) ♀
Controls	0/5	0/5	278	182	291	190	+ 13	+ 8
0.1% NaF	0/5	0/5	290	201	288	207	- 2	+ 6
0.2% NaF	0/5	0/5	264	188	237	183	- 27	- 5
0.4% NaF	2/5	2/5	278	185	211	144	- 67	- 41

Sodium fluoride is uniformly lethal to rats when incorporated in the diet at a level of 0.4 per cent or greater. At 0.2 per cent there is growth depression and an occasional death among female rats.

Problem Code: F.4 (Fate)

Section Code: 3210, 3220

Fluoride Metabolism:

Newburgh Study: The data reported previously in this study have been extended to include a larger number of control (Rochester) non-fasting samples. The results of the study are summarized as follows:

1. The mean blood fluoride level, for non-fasting samples of a community whose water supply contains approximately 1.3 ppm F is significantly higher (by a factor of 2.8) than that of a community whose water supply contains approximately 0.06 ppm F. There is considerable overlapping of individual samples from the two groups, however.

2. There is a significant difference between the means of fasting and non-fasting blood samples drawn from the same population.

3. The mean urinary fluoride levels from these two communities are in reasonably good agreement with the level of fluoride in the community water supply. Considerable variation about the mean exists among the individual samples, however, at the level of 1.36 ppm F in the water. A summary of the data is shown in Table 1 (Page 73).

After declassification these data were presented in the form of a paper at the 26th General Meeting of the International Association for Dental Research.

Kidney Dysfunction Studies:

Data relative to the excretion of fluoride by the normal and abnormal human kidney has been extended to include a patient afflicted with chronic glomerulonephritis. A summary of the data obtained to date is presented in Table 2 (Page 73), which indicates that the measurement of urinary fluoride

Table 1. Summary of Data From Newburgh Study

Sample	Mean Fluoride Level, ppm F	
	Rochester	Newburgh
Water supply	0.06	1.36
Urine	0.06	1.12
Non-fasting blood	0.014	0.04
Fasting blood	0.003	-

Table 2. Effect of Kidney Dysfunction on Urinary Excretion of Fluoride

Condition of Kidney	Per Cent Recovery in Urine of Fluoride Added to Drinking Water
Normal	48.4
Normal	46.0
Chronic Glomerulonephritis*	19.6
Pyelonephritis with hypertension	0.0

* No renal insufficiency

may be a clinical index of renal function since the damaged kidney appears either not to excrete ingested fluoride or to excrete decreased amounts from normal.

An investigation of the ability of the uranium damaged kidney to excrete fluoride has been initiated, using rabbits as the experimental animal. The data available to date indicate that the urinary excretion of fluoride by those animals which had received a single subcutaneous injection of uranium nitrate decreased as the damage to the renal tissue increased; the excretion then returned to its original level as the damage was repaired. The minimal excretion levels noted ranged from 7 to 23 per cent of the quantities being excreted prior to the injection of the uranium nitrate.

A second finding in this study suggests that fluoride may possess the ability to protect against the toxic action of uranium. It was found that two of four animals given the uranium injection only, died shortly after administration of the uranium, whereas no mortality was obtained in the four animals receiving 15 ppm fluoride in the drinking water and a single subcutaneous injection of uranium. Blood urea nitrogen determinations also support the protection hypothesis, since the urea nitrogen levels of the animals receiving only the uranium showed a two-fold increase over the urea nitrogen levels of those rabbits receiving both uranium and fluoride. Further work is being planned to corroborate and extend these findings.

Urinary Fluoride Output in Beryllium Pneumonitis:

The urinary output of fluoride added to the drinking water of three patients hospitalized with beryllium pneumonitis has been determined for periods of 15 to 30 days. Definite conclusions cannot be drawn regarding the efficiency of the urinary excretion of the added fluoride until

sufficient control data for comparable periods are available from normal individuals. Such data are being collected.

Blood Fluoride Levels Following Exposure to Hydrogen Fluoride:

A preliminary investigation of blood fluoride levels following exposure to hydrogen fluoride has been completed. In this study rabbits were exposed to approximately 29 mg. HF/m^3 for intervals of 6 to 30 hours; blood fluoride levels were then determined after each day of exposure, and for several days after termination of the exposure. The results indicate that exposure to hydrogen fluoride at a concentration of 29 mg. HF/m^3 produces a definite increase of fluoride in the blood, a ten-fold increase above the normal being noted in several of the animals. The levels are still abnormally high at least three days after the animals have been removed from the hydrogen fluoride atmosphere; normal values are again found six days after termination of the exposure.

Bone Metabolism:

The mechanism of fluoride fixation by bone has been investigated. Under proper experimental conditions, fluoride ion undergoes ionic exchange with hydroxyl ions. This exchange is uninfluenced by variations in the concentration of calcium and phosphate, is inhibited by an increase in hydroxide or bicarbonate concentration.

PROGRAM S.M.

SPECIAL MATERIALS

Problem Code: S.M.5 (Mechanism of Toxic Effect)

Section Code: 3210

Acute Toxicity of Zirconium by Injection:

Zirconyl nitrate, (Baker's Technical Grade, $ZrO(NO_3)_2 \cdot 2H_2O$) when injected intraperitoneally into mice (groups of 4 per dosage level) has given an LD50 roughly of the order of 450 mg/kg. The concentration of the solution injected was 50 mg/cc. in H_2O.

PROGRAM I.S.

ISOTOPES

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

Section Code: 3120

Studies of Protein Metabolism in the Dog Using C^{14} Labeled DL-Lysine. I. The Distribution of C^{14} Activity 24 Hours After Ingestion of ϵC^{14} Labeled DL-Lysine:

Background: As a result of earlier studies by Schoenheimer et al (1) it was concluded that the essential amino acid L-Lysine was incorporated into the tissue and blood proteins of the rat without undergoing transamination and without exchanging the δ, ν, or hydrogen atoms of its carbon chain. This has been referred to as the metabolic inertness of lysine and makes isotopically labeled lysine a useful reagent for labeling proteins and for studies of protein metabolism.

Helmkamp et al (2) have synthesized and made available for our use DL lysine with C^{14} in the ϵ carbon. With this compound it is possible to study not only the fate of the lysine molecule as a whole, but also to detect conversion of the lysine carbon chain to other intermediary metabolites.

Detailed data on the distribution of labeled lysine in the various tissues and blood protein fractions have not been obtained. It is the purpose of this study to obtain such data.

(1) Schoenheimer, R., et al. J. Biol. Chem., 140:779 (1941).

(2) Helmkamp, R., et al. J. Amer. Chem. Soc. In press.

Method: A dose of ζ C^{14} labeled DL-lysine was fed with a small amount of lean meat to a normal adult dog that had fasted for two days previously. The dog was then placed in an air-tight chamber through which a continuous stream of room air was drawn.

A carbon dioxide absorption apparatus was arranged so that every 2 hours all CO_2 in the air from the chamber could be absorbed in strong potassium hydroxide for a period of 30 minutes. Twelve CO_2 samples were obtained in this way.

The dog remained in the chamber for the 24 hours of the experiment except for brief intervals of 5-10 minutes at the close of CO_2 collection periods for the removal of blood samples drawn from the external jugular vein.

At the end of 24 hours, under light ether anesthesia, the dog was exsanguinated, and perfused with Locke's solution to remove residual blood from the tissues.

All viscera were removed, care being taken to avoid contamination with urine or bowel contents. Tissues were preserved by freezing and refrigeration, and prepared for assay by heat drying, vacuum dessication, or lysophiling (brain, lung, liver, kidney, thigh muscle, and temporal muscle). Lysophiling gave the most satisfactory tissue preparations.

Where albumin and globulin fractions were studied, the plasma was treated with 26.8 per cent sodium sulfate according to the procedure of Majoor (4); this procedure precipitates all globulins and leaves only albumin in solution. Other aliquots of plasma were treated with appropriate

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(4) Majoor, C. L. H. J. Biol. Chem., 169:583 (1947).

volumes of saturated sodium chloride to effect half saturation with sodium chloride and thus precipitate fibrinogen.

Chemical albumin to globulin ratios were determined by Kjeldahl analysis of aliquots of the same plasma and albumin solutions that were used for C^{14} assay. The globulin precipitates were assayed for C^{14} directly.

Aliquots of the expired CO_2 in potassium hydroxide were converted to barium carbonate and assayed for C^{14}.

All C^{14} assays were carried out after wet combustion of samples by the method of Folch and Van Slyke (5) by using the apparatus developed by Bale.

Results: I. The Distribution of C^{14} Activity 24 Hours after Ingestion of C^{14} Labeled DL Lysine:

The dose fed contained 6.00×10^3 volts/min.* activity.

A. The 24-hour urine was found to contain a total activity of 1970 volts/min. or 33 per cent of the dose fed.

B. The activity of the expired CO_2 rapidly increased after feeding to a maximum at about 12 hours later, then was maintained, dropping significantly in the last of the twelve 2-hour periods. (See Figure 1, Page 80). The total activity of the expired CO_2 was estimated by multiplying the total activity of the 30-minute samples by 4;

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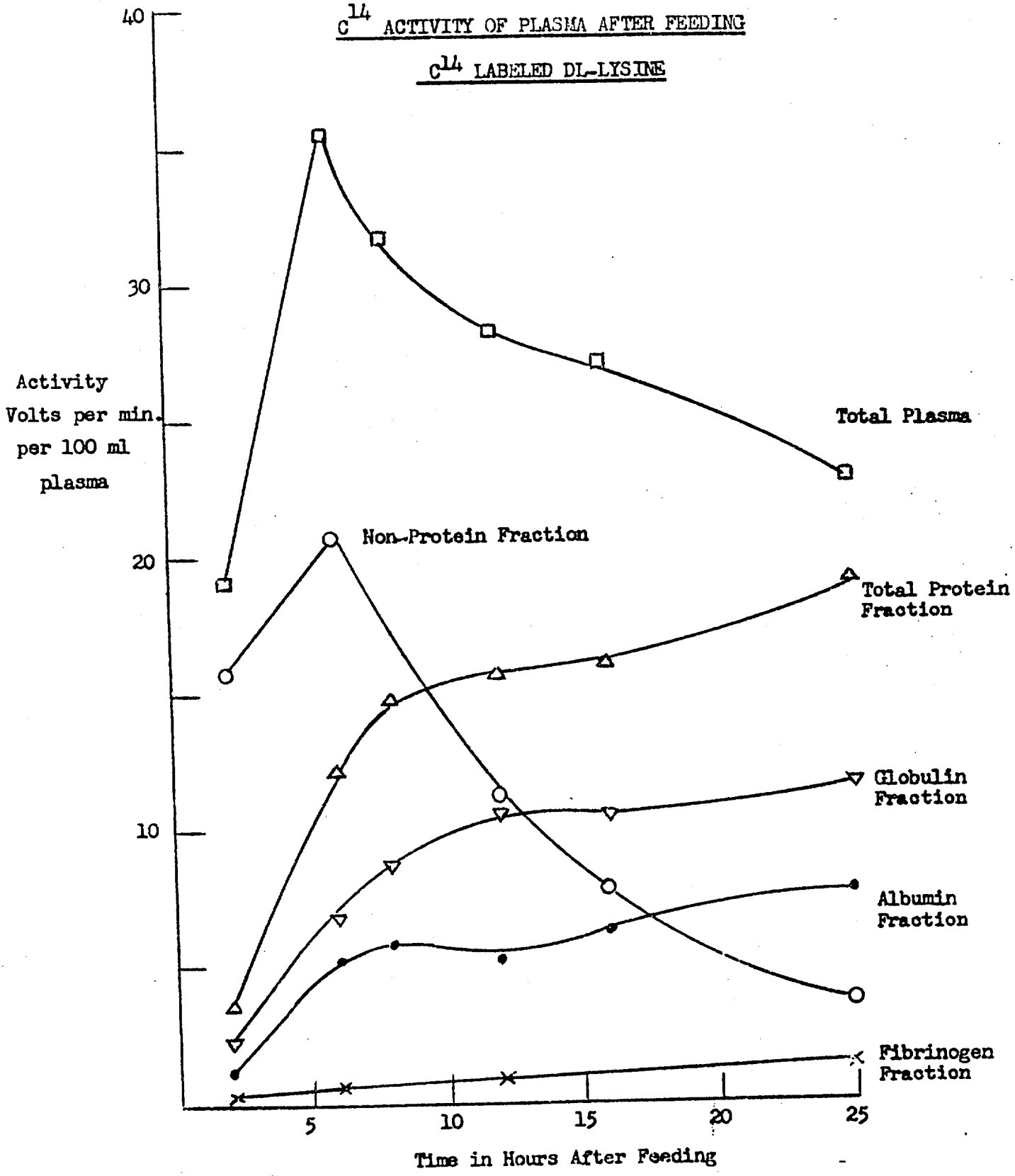
(5) Van Slyke, D. D. and Folch, J. J. Biol. Chem., 136;509 (1940).

* (Volts/min)x(1.88×10^4) = disintegrations/min. (Volts/min) x (8.46×10^{-3}) = microcuries.

Figure 1



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4 (420.8) = 1675 volts/min.

This corresponds to: $\frac{1675 \times 100}{6.00 \times 10^3} = 32.7\%$ of the dose fed

C. The C^{14} activity of the blood plasma, and the distribution of activity in non-protein, albumin, globulin, and fibrinogen fractions. (See Figure 1, Page 80).

1. The total C^{14} activity reached a peak of 35.5 volts/min/100 ml. plasma in the 6-hour specimen. Assuming a plasma volume of 500 ml., the maximum activity in the plasma corresponds to

$\frac{5\ (35.5)}{6 \times 10^3} \times 100 = 2.96\%$ of the total dose fed.

2. The C^{14} activity of the "non-protein" fraction reaches a peak of 20.8 volts/min/100 ml. plasma in the 6-hour specimen and drops to 3.6 volts/min/100 ml. plasma in the 24-hour specimen. Thus, at the close of the experiment, the activity in the non-protein fraction was:

$\frac{3.6}{22.7} \times 100 = 15.8\%$ of the total plasma activity.

3. The C^{14} activity of the total plasma protein rises rapidly (while the non-protein fraction's activity is rising) to 12.2 volts/min/100 ml. plasma and then rises more slowly after the first 6-hour to attain a maximum activity of 19 volts/min/100 ml. plasma at 24 hours, corresponding to 84.2 per cent of the total plasma activity and $\frac{500\ (19)}{6.0 \times 10^3}\ (100) = 1.58\%$ of the total activity fed.

4. The C^{14} activity of the albumin fraction rises similarly to 5.3 volts/min/100 ml. plasma at 6 hours, and then to a maximum of 7.5 volts/min/100 ml. plasma at 24 hours. This corresponds to:

$$\frac{5.3}{12.2} \times 100 = 43.5\% \text{ of protein activity at 6 hr.}$$

and

$$\frac{7.5}{19.0} \times 100 = 39.5\% \text{ of protein activity at 24 hr.}$$

when the C^{14} activity of the albumin fraction $\frac{7.5}{2.28} = 3.28$ volts/min/gm.

5. The C^{14} activity of the globulin fraction rises similarly to 6.8 volts/min/100 ml. plasma at 6 hours and more gradually to 11.5 volts/min/100 ml. plasma at 24 hours. This corresponds to:

$$\frac{6.8}{12.2} \times 100 = 55.7\% \text{ of total protein activity at 6 hr.}$$

and

$$\frac{11.5}{19.0} \times 100 = 60.5\% \text{ of total protein activity at 24 hr.}$$

and the C^{14} activity of the globulin fraction $\frac{11.5}{4.91} = 2.35$ volts/min/gm. In terms of C^{14} activity, the albumin to globulin ratio is:

$$\frac{43.5}{55.7} = 0.77 \text{ at 6 hr.}$$

and

$$\frac{39.5}{60.5} = 0.65 \text{ at 24 hr.}$$

Chemically determined albumin to globulin ratios from the same specimens.

Total protein at 6 hr. = 6.34 gm/100 ml. plasma

Albumin at 6 hr. = 1.73 gm/100 ml. plasma

$\frac{1.73}{(6.34-1.73)}$ = 0.38 at 6 hr.

Total protein at 24 hr. = 7.19 gm/100 ml. plasma

Albumin at 24 hr. = 2.28 gm/100 ml. plasma

$\frac{2.28}{(7.19-2.28)}$ = 0.47 at 24 hr.

6. The C^{14} activity of the fibrinogen fraction rises from 0.5 volts/min/100 ml. plasma at 6 hr. to about 1.25 volts/min/100 ml. plasma at 24 hr. Since the plasma at the termination of the experiment contained 0.36 gm. fibrinogen/100 ml., the C^{14} activity/gm. fibrinogen equals $\frac{1.25}{.36}$ = 3.48 volts/min/gm.

D. The C^{14} distribution in tissues expressed in terms of volts/gm. dried tissue and volts/min/gm. carbon is shown in the following incomplete list:

	Volts/min/gm. dried tissue	Volts/min/gm. carbon
Duodenum	1.86	3.10
Jejunum	2.04	3.10
Liver	1.54	3.20
Kidney	1.49	2.86
Lung	8.62	1.79
Lumph Node (cervical)		1.34
Thigh Muscle	.665	1.25
Brain	.286	0.495

Discussion: The fed C^{14} labeled DL lysine was almost completely absorbed from the gastro-intestinal tract and rapidly incorporated into the various blood and tissue proteins. The finding of highest activities in the blood plasma proteins, small intestine, liver, and kidney is very similar to previously reported observations with other amino acids such as glycine and methionine in the rat. It is of interest that the brain which has been supposed not to metabolize amino acids other than glutamic, has an appreciable C^{14} activity (mainly as lysine).

The large proportion of fed C^{14} activity in the urine is accountable on the basis of the known fact that the unnatural D-isomer of lysine is not utilized by the organism as such, and when fed, is excreted in large measure unchanged. This is in agreement with the observation of Schoenheimer et al (See Reference (1), Page 77), that D Lysine labeled with N^{15}, fed to rats, is mainly excreted in the urine unchanged.

The very early appearance of C^{14} activity in the expired CO_2 is a good index of the rapidity with which oxidation of the fed amino acid ensues; a large portion of this activity is probably derived from the oxidation of the D Lysine. The average maximal specific activity of the expired CO_2 per millimole was of the order of 0.5 volts/min.

The C^{14} activity of the blood plasma proteins attained a maximum activity at the close of the experiment corresponding to 1.58 per cent of the total activity fed, or to about 3.2 per cent of the fed dose in terms of the natural isomer. The chemical albumin to globulin ratio is approximately one-half the C^{14} albumin to globulin ratio at 6 hr. and two-thirds the C^{14} albumin to globulin ratio at 24 hr. This may be interpreted to indicate that more lysine is incorporated in 1 gm. of albumin than in 1 gm. of globulin and may simply be a reflection of the fact that serum albumin has

approximately twice the lysine content of serum globulin. The shift downward in the C^{14} A/G ratio at 24 hr. implies that (ϵC^{14} lysine labeled) albumin molecules are produced at a lesser rate than globulin, or expressed otherwise that the mean globulin turnover is more rapid than albumin turnover. This latter view is supported by the apparently more rapid disappearance of globulin C^{14} than albumin C^{14} from the plasma of the normal dog injected with C^{14} labeled plasma proteins. (See "Results II", Page 86).

It is of interest that at 24 hr. after feeding the DL lysine, the fibrinogen fraction has an activity of 3.48 volts/min/gm. which is about the same as that of the albumin fraction. Because the earlier fibrinogen fractions had such low activities, it is not feasible to compare them with the albumin or total globulin fractions at this time.

Summary:

1. DL-lysine labeled in the ϵ carbon with C^{14} has been fed to a normal dog and the distribution of activity in expired CO_2, urine, blood, and tissue proteins studied.

Studies in Protein Metabolism in the Dog Using C^{14} Labeled DL-Lysine. II. The Fate of C^{14} Labeled Plasma Proteins When Injected Intravenously Into A Normal Dog:

Background: The study of Fink, Enns, et al (1) made use of fed N^{15} labeled lysine to produce labeled plasma proteins in a normal dog, and used the resulting plasma for injection into normal dogs and dogs suffering from shock incidental to intestinal trauma. In these experiments it was found that the labeled proteins disappeared rapidly in non-logarithmic

(1) Fink, R. M., Enns, T., et al. J. Exp. Med., 80:455 (1944).

fashion with about 50 per cent gone from the plasma at 24 hr. and about 64 per cent gone at 48 hr. This was similar to the observations of Fine and Seligman (2), using radioactive sulfur labeled cystine in plasma proteins, who reported that 30 per cent of injected plasma was gone from the circulation in 15 hours and 55 per cent was gone at 48 hours. Likewise, Heidelberger et al (3), has shown that rabbit serum antibody protein injected into a rabbit was removed from the circulation to the extent of 38 per cent at 23 hr. and 56 per cent at 48 hr.

None of the above studies attempted to compare the extent of incorporation of the isotope in the several plasma protein fractions after feeding or the extent of disappearance of these labeled fractions when injected with whole plasma.

Method: A large portion of the blood from the dog fed C^{14} labeled lysine was heparinized, and the plasma was given intravenously to a normal recipient dog, to allow a study not only of the disappearance of the labeled plasma as a whole, but also the relative disappearance of the serum albumin fraction as compared with the total globulin fraction. Blood samples were drawn at intervals from the recipient dog for protein fractionation and C^{14} assay.

Methods of protein fractionation and C^{14} assay were the same as those used in Experiment I. (See Page 78).

Results: The graph (Figure 2, Page 87) shows that 30 minutes after the injection of the C^{14} labeled plasma the activity as measured directly on the plasma was 4.3 volts/min/100 ml. of whole plasma; 24 hours later,

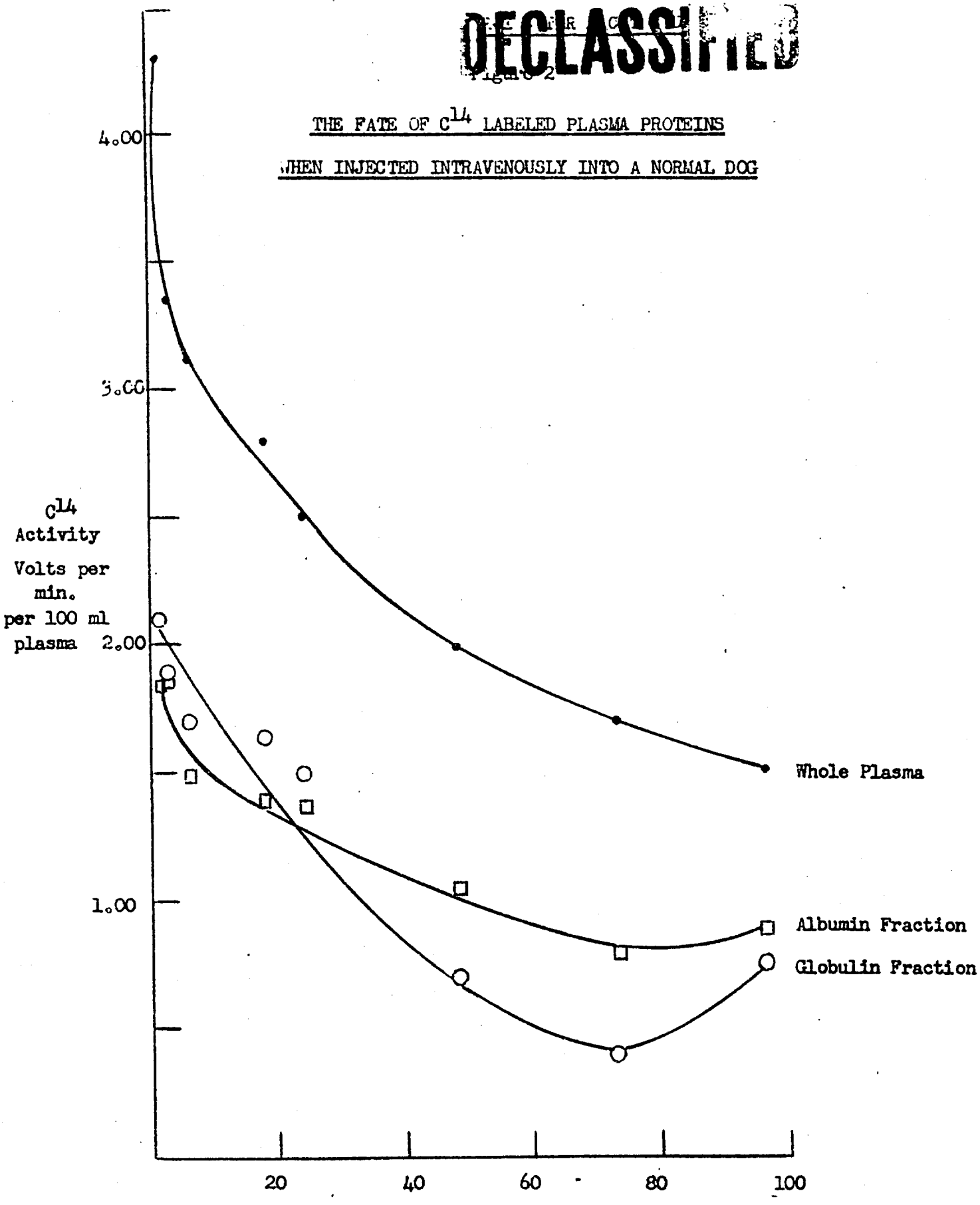
(2) Fine, I. and Seligman, A. M. J. Clin. Inv., 22:285 (1943).

(3) Heidelberger, M. et al. J. Biol. Chem., 144:555 (1942).

Figure 2

THE FATE OF C^{14} LABELED PLASMA PROTEINS

WHEN INJECTED INTRAVENOUSLY INTO A NORMAL DOG



the activity was 2.5 volts/min/100 ml., and 48 hr. later, the activity was 2.0 volts/min/100 ml. The "half-life time" of the total plasma on this basis is approximately 40.5 hr. These data were not corrected for dilution of activity consequent to the removal of plasma in the course of sampling.

The "half-life" of albumin C^{14} activity was approximately 57 hours, and that of the globulin C^{14} activity was approximately 37 hours.

Discussion: It is significant that the globulins as a group appear to be removed more rapidly from the plasma than the albumin fraction. This may be a reflection of the greater ease with which the organism can elaborate and turn over plasma globulins as compared with albumin.

Summary: Intravenously injected C^{14} lysine containing plasma proteins disappear rapidly from the circulating plasma of a normal dog with the globulin fraction half gone at about 37 hours and the albumin half gone at about 57 hours after injection.

Studies in Protein Metabolism in the Dog Using C^{14} Labeled DL-Lysine. III. The Fate of C^{14} Labeled Plasma Proteins When Injected Into the Peritoneal Cavity of the Experimentally Ascitic Dog:

Background: Ascitic fluid has long been known to contain large amounts of protein which undoubtedly comes from the circulating plasma and consists largely of albumin.

McKee and Schilling (1) have devised a simple technique of inducing ascites by surgically obstructing the inferior vena cava above the diaphragm in the dog.

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(1) McKee, F. W. et al. Fed. Proc., 6:396 (1947).

The exchange of the protein in ascitic fluid with that in the plasma has not hitherto been studied. The availability of C^{14} labeled plasma proteins from a normal donor animal makes possible such a study.

Method: A large volume of C^{14} labeled heparinized plasma was injected into the peritoneal cavity of an ascitic dog to determine the rate of disappearance of proteins from the peritoneal cavity into the blood in ascites. Samples of blood were drawn at intervals up to 48 hours at which time the peritoneal cavity was tapped. The fluid removed was measured and then assayed for residual C^{14} activity.

Results: The graph (Figure 3, Page 90) shows that C^{14} activity appeared in the circulating plasma in detectable amounts at 2 hours after the injection (1.9 per cent of total dose given), and reached 12.9 per cent of the total dose given at 48 hours. Since at 48 hours 32.5 per cent of the total activity given was still present in the ascitic fluid, about 56 per cent must have been removed from the blood.

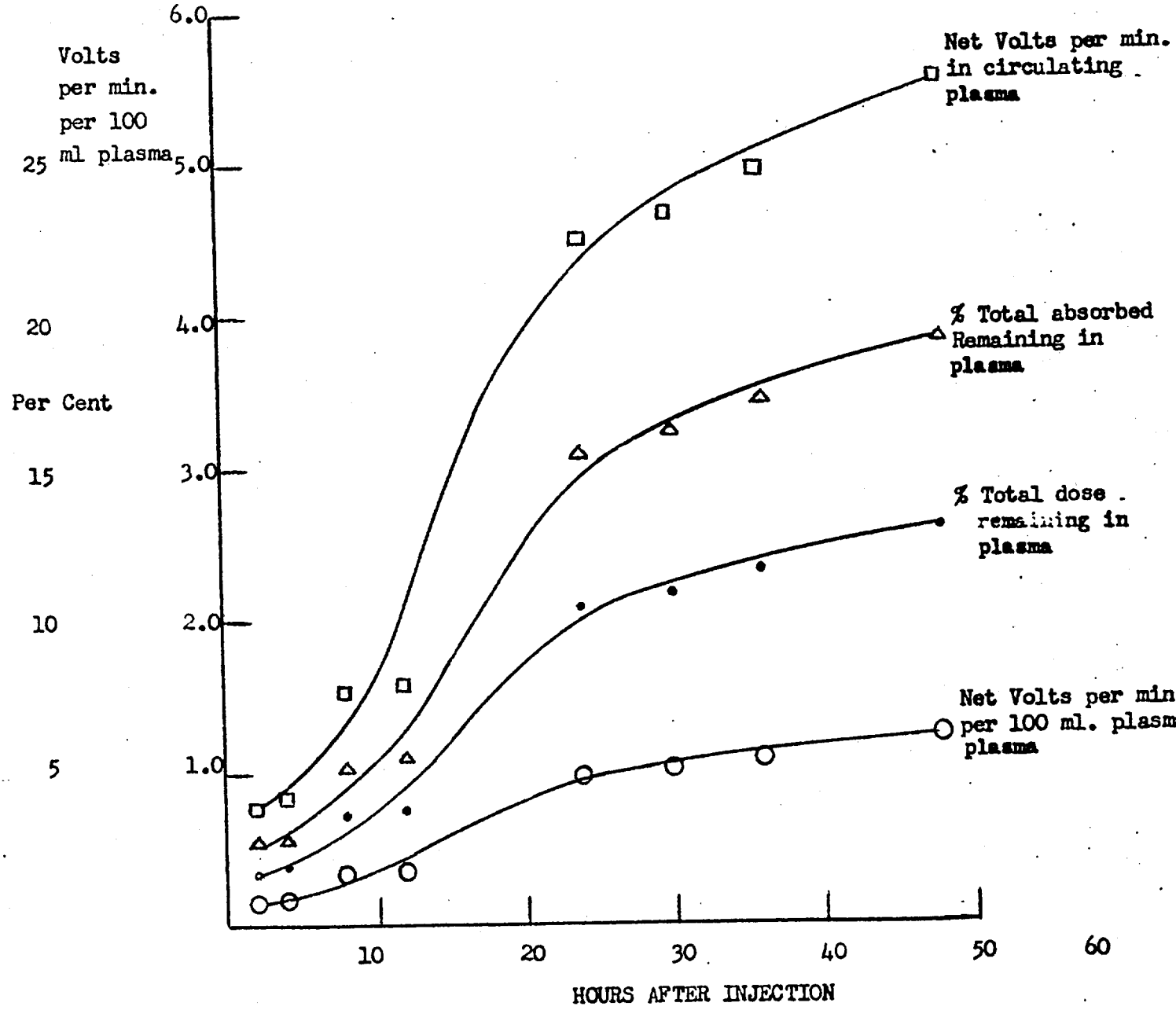
Since the C^{14} activity of the plasma rose only from 1.03 to 1.22 volts/min/100 ml. in the last 18 hours, and since the activity of the ascitic fluid was 2.0 volts/min/100 ml. at 48 hours, a steady state was being approached in which activity was being removed from the plasma as rapidly as it was entering the plasma.

Discussion: The rapid appearance of labeled plasma proteins in the circulating plasma of an ascitic dog after these proteins were injected into the peritoneal cavity indicates that in the process of ascites formation, the protein components of the apparently stagnant accumulation in the peritoneal cavity exchange rapidly with the circulating plasma proteins, and thus represent but another demonstration of the dynamic relation between the

Figure 3

ABSORPTION OF C^{14} LABELED PLASMA

FROM PERITONEAL CAVITY IN ASCITES



Dose Injected = 130 ml plasma (.33v/m/ml) = 43 v/min.

Dose Absorbed = 43 (1400 x .020) = 43-14 = 29 v/m

Ascitic Fluid = 1400 ml. volume

circulating plasma proteins and other proteins in the body. Because of the low activities, it was not feasible to compare the C^{14} albumin to globulin ratios of the circulating plasma with those of the injected plasma, to ascertain whether the albumin exchanges more rapidly than the globulin in the ascitic fluid.

Summary: Injected C^{14} lysine labeled protein components of ascitic fluid in canine experimental ascites are in dynamic exchange with the plasma proteins in spite of continuous increasing accumulation of ascitic fluid.

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

IV. The Intermediary Metabolism of Lysine:

Background: The intermediary metabolic fate of the carbon chain of lysine is not known, although a brief note by Borsook et al (1) reports the conversion of C^{14} labeled lysine to α-aminoadipic acid by guinea pig liver slices in vitro.

The availability of tissues from a normal dog, sacrificed 24 hours after ingestion of C^{14} labeled DL-lysine, has made possible a chemical study of the amino acids isolable from tissues. Pure chemical individuals can then be studied for C^{14} content and structural relation to lysine.

Method: For the isolation of amino acids, tissues were hydrolyzed with 20 per cent HCL; the dicarboxylic amino acids were removed from the resultant mixture with the anion exchange resin Amberlite IRA-4, and the basic amino acids by the cation exchange resin Amberlite IRC-50.

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(1) Borsook, H. et al. J. Biol. Chem., 173:420 (1948).

The lysine was then isolated as a styphnate, which was recrystallized, and then converted to crude lysine, .2HCL (contaminated with small amounts of arginine and histidine). The lysine, .2HCL was then converted to the picrate which was recrystallized and found to be free of contaminants on paper chromatography. A portion of the lysine picrate was reconverted to lysine .2HCL for chemical characterization.

The dicarboxylic amino acids were isolated as glutamic acid hydrochloride and copper aspartate by accepted methods.

Results:

The Chemical Identification of the Radioactive Metabolites in Tissues.

The method of separation used in this study revealed that C^{14} activity was present in the basic amino acid fraction (arginine, histidine, and lysine), in the dicarboxylic amino acid fraction (glutamic acid and aspartic acid), and also in the monoamino-monocarboxylic acid fraction. The findings for liver were typical:

A. The Basic Amino Acid Fraction:

Lysine was isolated as the picrate and converted to lysine-2HCL. Both had proper chemical constants and showed no detectable amino acid contaminants on paper chromatography. The C^{14} activities of:

1 gram whole dried liver = 1.54 volts/min.

Basic fraction from 1 gm. whole dried liver = 0.930 volts/min.

Lysine equivalent to 1 gm. whole dried liver = 0.481 volts/min.

Basic fraction activity unaccounted for by lysine = 0.449 volts/min.

29% of total activity

The possibility that arginine may be derived from lysine and contains C^{14} is being investigated.

B. The Dicarboxylic Amino Acid Fraction:

Glutamic acid was isolated from liver as the hydrochloride and aspartic acid as copper aspartate. Both had proper chemical constants, and contained no detectable contaminating amino acids by paper chromatography. Since 38.6 mgs. glutamic acid hydrochloride from the liver had .084 volts/min. activity, then the activity in 1 gm. of liver (10.2%N) may be estimated by assuming that liver protein (16%N) contains 11.4% glutamic acid (Block) (2).

$$\frac{10.2\ (6.25)\ (11.4)}{38.6\ (.8)} \times (.084) = 0.198 \text{ volts/min/gm. dried liver}$$

This corresponds to $\frac{0.198}{1.54} \times (100) = 12.8\%$ of total activity in liver and on a molar basis, the glutamic acid has 16.7% of the activity in the liver lysine computed as follows:

$$\frac{220}{16.1} \times (0.175) = 2.39 \text{ volts/min/millimol of lysine}$$

$$\frac{(183.5)}{38.6} \times (.084) = 0.40 \text{ volts/min/millimol of glutamic acid}$$

$$\frac{.40}{2.39} \times (100) = 16.7\%$$

Since 31.8 mgs. of liver copper aspartate had 0.018 volts/min. activity, then the activity in 1 gm. of dried liver (10.2%N) may be estimated by assuming that liver protein (16%N) contains 7.4% aspartic acid. (Block) (2).

(2) Block, R. J. "The Amino Acid Composition of Proteins and Foods", C. C. Thomas, Springfield, Illinois, 1945.

$$\frac{10.2\ (6.25)\ (7.4)}{31.8\ (.673)} \times (0.018) = .040 \text{ volts/min./gm. liver}$$

This corresponds to:

$$\frac{.04}{1.54} \times (100) = 2.6\% \text{ of total activity in liver}$$

On a molar basis, aspartic acid has 4.6% of the activity in liver lysine, and 27.5% of the activity in liver glutamic acid computed as follows:

$$\frac{194.7}{31.8} \times (0.018) = .11 \text{ volts/min/millimol aspartic acid}$$

$$\frac{.11}{2.39} \times (100) = 4.6\%$$

$$\frac{.11}{.40} \times (100) = 27.5\%$$

The moncamino-carboxylic acid fraction by paper chromatography contains less than 5% of the original basic amino acids, and less than 10% of the dicarboxylic amino acids, yet the C^{14} activity in the monoamino-carboxylic acid fraction of the liver has .171 volts/min/gm. or $\frac{.171}{1.54}$ (100) = 11.1% of the total activity present. The identification of the C^{14} active components is being actively pursued.

Discussion: The most significant general observation made in the study of isolated amino acids indicates that the carbon chain of lysine is definitely converted to other amino acids. Because the specific activity of the liver glutamic per millimol is of the same order of magnitude as that of the expired CO_2, it is not possible that it was derived from the CO_2 assimilation reaction of Wood and Werkman (3). In this

(3) Wood, H. B. and Werkman, C. H. Biochem. J., 30:48 (1936).

reaction, only 1 millimol of CO_2 would be used to form 1 millimol of glutamic acid, and the resultant dilution by inert glutamic acid of tissues would give glutamic acid of activity several hundred times less than that observed. By the same token, the activity observed in the liver aspartic acid was also very probably derived from the lysine via glutamic acid.

We have no evidence for the occurrence of α-aminoadipic acid as a breakdown product of lysine, although this was reported by Borsook et al (See Reference (1), Page 91) to result from the in vitro oxidation of L-lysine by guinea pig liver. It is conceivable, however, that α-aminoadipic acid may be an intermediary metabolite in the conversion of lysine to glutamic acid, but that the concentration of α-aminoadipic acid under physiological conditions is very low and has escaped detection by paper-chromatographic methods used which would have detected as little as 0.1% of α-aminoadipic acid in the dried liver.

Because the lysine activity of the liver basic amino acid fraction does not account for all activity present, the amino acid arginine is being examined for possible activity. It is known that the adult dog can elaborate enough arginine from unknown precursors to maintain itself in nitrogen balance without dietary arginine. The possibility that the lysine carbon chain may serve as an arginine precursor is therefore worthy of consideration.

The occurrence of activity in the monoamino monocarboxylic amino acid fraction is surprising, but the possibility of oxidative splitting of the lysine carbon chain and conversion to short chain amino acids such as alanine remains to be ruled out.

Summary:

1. Glutamic and aspartic acids have been isolated from the liver

of a normal dog fed ϵ-C^{14} labeled lysine, and their activity leads to the conclusion that lysine may serve as an immediate metabolic precursor of these amino acids.

2. L-lysine has been isolated from the tissues of a normal dog fed ϵ-C^{14} labeled lysine, and the total lysine activity does not account for the total activity of the corresponding basic amino acid fraction.

3. The monoamino monocarboxylic amino acid fraction of tissues from a normal dog fed ϵ C^{14} labeled lysine contains enough activity to suggest the possibility of conversion of the lysine carbon chain to that of shorter chain amino acids such as alanine.

Problem Code: I.S.2 (Radioautography)

Section Code: 3220

Partition-Chromatograph of Lipids:

An apparatus has been constructed which permits the analysis of the radioactivity of paper chromatograms.

PROGRAM H.P.

HEALTH PHYSICS

Problem Code: H.P.1 (Research and Development)

Section Code: 3320

Investigation of Film Behavior: Preliminary Report on the Wavelength Dependence of a Photo-Sensitive Emulsion in Terms of Densities Recorded for Known Radiation Intensities:

Background: Photographic emulsions are being used as radiation indicators in the form of pocket meters, finger rings and wrist bands. Since it appears that in general only superficial knowledge is available pertaining to the response of these emulsions to a variety of radiation wavelengths, it was deemed advisable to explore these responses in greater detail.

Methods: In order to set a limit for a range of wavelengths most likely to occur in practice, it was decided to start this range at x-ray wavelengths produced by .05 MEV and to extend this range to radiation of 2.0 MEV x-rays. This choice should cover the wavelengths range normally found in common radio-isotopes. The available x-ray generating equipment for this investigation consisted of (1) a .14 MEV generator, (2) a .25 MEV generator, and (3) a 1 MEV generator. (Provisions are now being made to obtain the use of a .4 and 2 MEV generator.)

The procedure consisted of many controlled exposures of a single type of x-ray film, commonly employed in monitoring services. This limitation to a single type of photographic emulsion was based on the reasoning that all pilot experiments would provide better control over the basic discrep-

ancies to be expected in any photo-sensitometric experiments. Control of radiation intensity was achieved by simultaneous measurements of these intensities by an ionization meter during the film exposures; a Victoreen r meter, calibrated by the U. S. National Bureau of Standards, constituted the measuring device. For x-rays produced by energizing voltages of .05, .10, .15, .20, and .25 MEV correction factors were applied to the meter readings from the Bureau's standardization. These correction factors indicate the different behaviors of the chambers at different x-ray spectra. (A free air ionization chamber does not reveal this discrepancy in the same order of magnitude and such equipment is now available at this Project.) Further corrections had to be made for changes in atmospheric pressure and temperature during exposures of the chambers and emulsions in order to reduce all values to normal pressure and temperature.

Since it became necessary to employ several pieces of x-ray generating equipment in order to cover the range of x-ray wavelengths, it was decided

thus renders the results universally applicable.

Experimental exposures were performed by two methods: (a) film packets were exposed to individually calculated exposure series arranged in algebraic progression, and (b) single exposures through a sensitometer disc, which permitted exposures of geometric progression.

Development of all exposed emulsions was performed in a specially designed lucite rack which permitted uniform control during development by mechanical agitation. With each series of films a sensitometric control strip was developed in order to correlate all development processures.

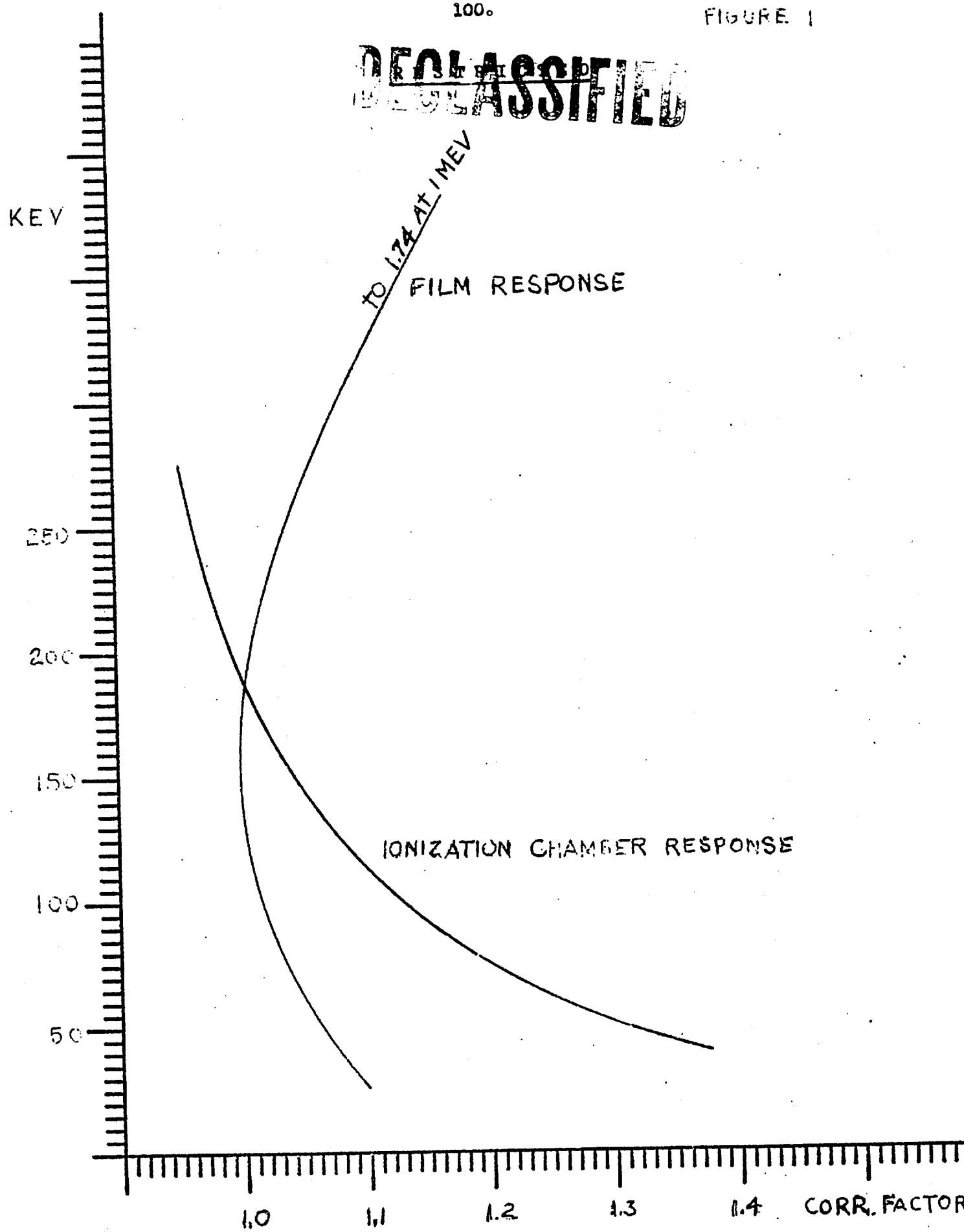
The preliminary findings have been plotted in Figure 1 (Page 100) and can be interpreted as follows:

(1) That the radiation intensities as measured by the ionization chamber result in film densities varying with wavelength ranges.

(2) That these variations cannot be simply correlated with similar changes indicated by ionization chamber measurements.

The film response curve is plotted in Figure 1 for a constant density of 2.0 in terms of correction factors versus x-ray spectra produced by exciting potentials from 50 to 250 KEV. These correction factors are obtained by relating all exposures to the minimum exposure at a certain wavelength range, which produces a film density of 2.0. Although values were obtained for 1.0 MEV x-rays, they have not been plotted on the graph since no intermediate values are available at this time. The same scale of correction factors is also employed for the ionization chamber response and merely indicates the essential differences in trend of the two radiation recording methods. It can be seen that for the specific film used in this preliminary study, maximum density appears at a radiation wavelength range of 150 KEV

FIGURE 1



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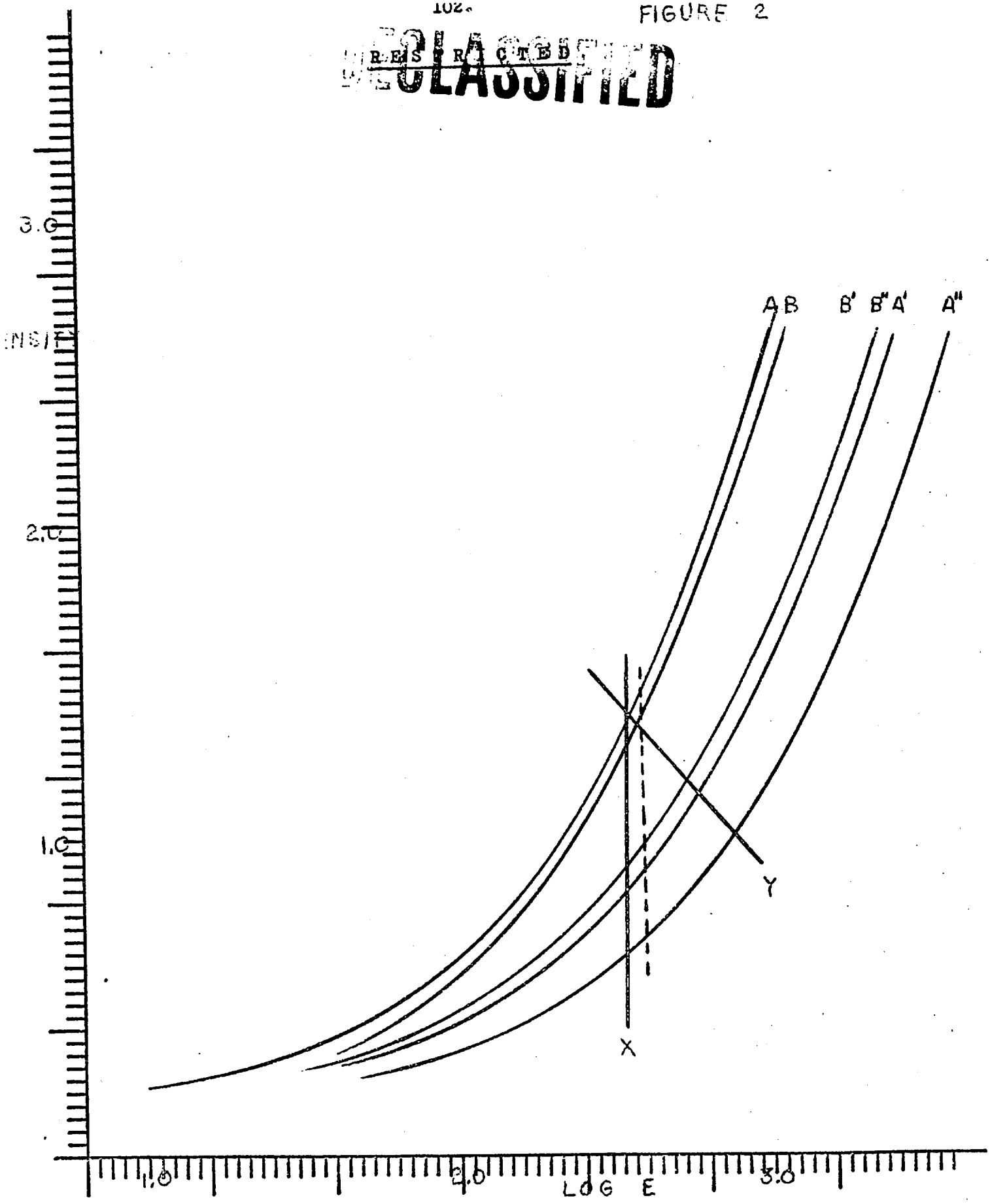
x-rays, and that the sensitivity of this film decreases apparently steadily as the radiation wavelengths are decreased. Determination of this maximum and investigation of the probable slope of the response constitutes one of the purposes of this study.

Since all of the preliminary work had to be done with x-radiation, it appeared that an interesting survey could be made parallel with the original experiments. This survey was extended to the Department of Radiology (Medical School) as a monitoring service for personnel operating x-ray generators. Film packets were distributed to this personnel for the detection of radiation quantities received during weekly operations. Equipped with two copper filters 0.12 and 0.24 mm. thick, three areas of different densities become available for interpretation and consequent translation into radiation quantities received by the wearer. The following method is employed for the correlation of these densities with standards. These standards are obtained by exposing films to known intensities and wavelengths ranges, thereafter plotting the developed densities against log exposure. In Figure 2 (Page 102) is shown a set of curves for illustration. Curves A, A', and A" show a series of densities obtained by exposing the above named film to x-radiation excited by 100 KEV. Densities of curve A are those measured on the portion of the film which received the direct radiation, and densities of curves A' and A" are those measured on the film under the .12 and .24 mm. Cu. filters respectively. The curves marked B, B', and B" were obtained by an exposure to 200 KEV x-radiation, and their relative positions again show direct exposure and filtered exposures through the same copper filters as above.

Application of these curves is illustrated as follows;

FIGURE 2



Case 1. Film densities as measured on a monitoring badge may have values of 1.42, 0.85, and 0.65. From Figure 2 it is seen that these points can be connected by a line "x" with zero slope. This means that the radiation received by the badge must have been similar to that which was used for the standard curves A, A', and A", namely, 100 KEV.

Case 2. Film densities measured on another monitoring badge may reveal values of 1.42, 1.16, and 1.03. These values would be found to lie on a connecting line "y" on a 100 KEV x-ray standard. However, this line, presenting a slope of approximately one, cannot be interpreted in terms of a single log E value, stating a specific single exposure value as was feasible in Case No. 1. It, therefore, becomes necessary to fit these density values to another set of standards until a match with standard densities has been achieved. For illustration purposes this was done by adding curves B, B', and B" on the same figure. These curves represent conditions for x-radiation produced by accelerating voltages of 200 KEV. In this example a reasonable fit of densities is possible as shown by the broken line with zero slope. The points of intersection of this line with curves B, B', and B" originate from a movement of similar density points on line "y" and curves A, A', and A" parallel to the abscissa. Identification of the radiation wavelength range of 200 KEV x-rays has been accomplished and a qualitative as well as quantitative interpretation results.

The method has been in effect for some time and is being investigated further for its merits as well as its faults. A simple slide rule computer is in the process of design and this should reduce time of interpretation considerably.

This preliminary report has been rendered purposely with a minimum

of data concerning complications and procedures, since many more factors must be investigated prior to any final report.

PROGRAM C.S.

SPECIAL CLINICAL SERVICE

Problem Code: none

Section Code: 3312

Introduction: Three additional patients with chronic pulmonary granulomatosis of beryllium workers were studied in the Metabolism Ward during the past three-month period. It is of interest that each of these three patients represents a single stage in the disease. Case #1 shows findings compatible with relatively early involvement, while Case #2 illustrates moderately advanced pathology, and Case #3 advanced involvement. Complete summaries of the case histories of these individuals are given, together with complete results of all laboratory findings, x-rays, respiratory changes, and nitrogen balance studies on a carefully controlled diet. It is of interest that these individuals showed evidence of not only pulmonary involvement but degrees of liver and kidney involvement as well. The significance of these findings, together with the new observations of impaired nitrogen absorption are discussed in considerable detail.

CASE #1:

Clinical History: This 46-year old patient was in excellent health until August, 1946. At that time, while on vacation in the mountains of Oregon, she developed a persistent tickling sensation in her throat and a hacking cough. Because her symptoms persisted, she finally went to her family doctor in March, 1947, who felt that she had mild sinusitis. In June, 1947 she returned to her home in Lorain, Ohio, and shortly thereafter

became aware of dyspnea on exertion. In October, 1947 she was told that her chest x-ray was "cloudy" and that it might be due to beryllium poisoning. Patient was referred here for study of this possibility. The cough was present particularly in the morning and at night and was productive of about 1 tablespoonful of mucoid sputum each day. The dyspnea, the patient feels, has been progressive and that even the mildest exertion, such as dusting a room, causes shortness of breath. There has never been any dyspnea at rest, no orthopnea, no paroxysmal nocturnal dyspnea. There have been no associated symptoms, no weight loss, anorexia, chills or fever or cardiac symptoms.

Pertinent to her present illness is her occupational history. In 1944 she was employed at a job analyzing beryllium powder. She worked at this for three months and stopped because of the development of a rash on her arms. She has had no other exposures to beryllium, and the onset of her symptoms occurred while she was on vacation in the west.

Past History: Non-contributory.

Physical Examination: Blood pressure 120/80. P 80, R 20. Patient was a well-developed and well-nourished 46-year old white female who appeared neither chronically nor acutely ill. Occasional hacking cough was observed; no respiratory distress. No cyanosis or clubbing. Head: normal. Eyes: negative. Ears: negative. Pharynx: clear. Neck: thyroid not enlarged, no venous engorgement. Lymph nodes: small discrete bilateral axillary nodes. Heart: LBCD 8 cm. from MSL in 5th interspace. Rhythm regular, sounds of good quality, no murmurs. P_2 is greater than A_2. Lungs: expansion good. Percussion note normal. There were scattered rhonchi in the right chest posteriorly, otherwise lungs were clear. Abdomen: no

organs felt. Extremities: negative. Pelvic: retroversion uterus. Rectal: negative.

Admission Laboratory Data:

Blood Count:

RBC 6.76 M.

Hgb. 11.8 gms.

WBC 4,500

Differential normal.

Platelets adequate.

Urinalysis: negative

Blood Chemistry:

Total proteins 6.5 gms.%

Albumin 4.1

Globulin 2.4

Blood urea nitrogen 19.8 mg.%

CO_2 55.5 volumes %

Chloride 101 meq.

Calcium 10.0 mg.%

Phosphorus 3.9 mg.%

Cephalin flocculation negative

Total bilirubin 6.8 mg.%

Glucose Tolerance Test: after 50 gm. glucose, p.o.

Fasting 42.5 mg.%

½ hour 118 mg.%

1 hour 149 mg.%

2 hours 88 mg.%

3 hours 78 mg.%

4 hours 76 mg.%

Insulin Tolerance Test: after .1 of a unit of insulin per kg. of body weight, i.v.:

Fasting 90 mg.%

20 minutes 21.6 mg.%

30 minutes 30.2 mg.%

45 minutes 34 mg.%

60 minutes 47 mg.%

90 minutes 70 mg.%

120 minutes 58 mg.%

Continuation of Insulin Tolerance Test: after 0.5 cc. of 1:1000 solution of adrenalin hydrochloride:

30 minutes 53 mg.%

60 minutes 94.5 mg.%

Vitamin A Absorption: after 7000 I.V. ester Vitamin A per kg. body weight:

Basal 9 Evelyn units (normal about 15-40)

4½ hours 309 (normal 250)

7 hours 366 (normal 140)

BSP test after 45 minutes negative (5 mg. of dye per kg.)

Urobilinogen .39 and .32 Erlich units per 2 hours

Urea Clearance 105% of normal

PSP test 35% excretion in 15 minutes

Electrophoresis report:

Total proteins 6.3

Albumin 3.3

Normal values of globulin constituents

Beryllium Analyses (Urine)

Case #1

Sample Date	Volume Used	Be Concentration
4/23-4/24/48	200 ml.	0.00 µg/liter
4/25-4/26	"	0.03
4/27-4/28	"	0.00
4/29-4/30	"	0.03
5/1-5/2	"	0.00
5/3-5/4	"	0.00
5/5-5/6	"	0.00
5/6-5/7	"	0.00
5/8-5/9	"	0.00
5/10-5/11	"	0.03
5/12-5/13	"	0.06
5/14-5/15	"	
5/16-5/17	pooled 600 ml.	0.00
5/18-5/19		
5/20-5/21	200 ml.	0.02
5/22-5/23	"	0.06
5/24-5/25	"	0.04
5/26-5/27	"	0.00
5/28-5/29	"	0.00

Respiratory Studies

The maximum breathing capacity was normal. The total pulmonary capacity was slightly reduced, and the vital capacity was only two-thirds of predicted value. Bronchospirometry revealed normal proportions of ventilation between the two lungs, yet the right lung was responsible for 67 per cent of the total oxygen uptake. The electrocardiogram was normal. Whereas the arm to lung circulation time was prolonged to 10 seconds, the arm to mouth circulation time and venous pressure were normal. By cardiac catheterization the right ventricular systolic pressure was fluctuating above the normal range during rest, and increased to the hypertensive range during moderate exercises with the legs. The cardiac output was normal. The arterial oxygen saturation was reduced to 85 per cent and the pO_2 to 75. Pulmonary efficiency in relation to work was below normal, yet patient was able to perform grade walking as high as 10 per cent incline for 5 minutes at the cost of moderate dyspnea and greatly increased oxygen debt.

In addition to impaired diffusion of oxygen permitting hypoxemia, this patient presented evidence of early cor pulmonale. She did not exhibit as marked compensatory hyperventilation as other patients, however.

Metabolic Studies

Nitrogen balance studies were also carried out on this patient for approximately one month and the results are as reported on this page and the following page.

Nitrogen Balance

Period*	Diet	Urine	Stool	Balance	Body Weight
2	14.57	11.02	1.40	+ 2.15	57.68
	14.57	13.45	1.40	— .28	

Nitrogen Balance (cont.)

Period*	Diet	Urine	Stool	Balance	Body Weight
	14.57	11.06	1.40	+ 2.11	
	14.57	10.80	1.40	+ 2.37	
	14.57	10.48	1.40	+ 3.04	
	14.57	10.13	1.40	+ 2.69	
				+12.08	
3	14.57	13.5	1.40	- .33	58.21
	14.57	12.85	1.40	+ .32	
	14.57	12.98	1.40	+ .19	
	14.57	13.18	1.40	- .01	
	14.57	12.38	1.40	+ .79	
	14.57	13.18	1.40	- .01	
				+ .95	
4	14.57	13.47	1.40	- .30	57.14
	14.57	13.4	1.40	- .23	
	14.57	14.74	1.40	-1.57	
	14.57	12.92	1.40	+ .25	
	14.57	14.0	1.40	- .83	
	14.57	9.82	1.40	+ 3.35	
				+ .67	
5	16.16	12.82	1.64	+ 1.70	57.72
	16.16	13.67	1.64	+ .85	
	16.16	13.43	1.64	+ 1.09	
	16.16	12.23	1.64	+ 1.29	
	16.16	13.99	1.64	+ .53	
	16.16	13.38	1.64	+ 1.14	
	16.16	13.75	1.64	.77	

Nitrogen Balance (cont.)

Period*	Diet	Urine	Stool	Balance	Body Weight
	16.16	13.76	1.64	+ .76	
	16.16	12.76	1.64	+ 1.76	
				+ 9.89	58

*Period 1 is not reported inasmuch as during this time the subject was approaching nitrogen balance on a control diet and had not reached a constant metabolic state.

Interpretation: This patient had not lost weight, was not poorly nourished at the start of the metabolic study. She remained in approximate nitrogen equilibrium throughout experimental period. It was noted that the stool nitrogen was not excessively high. This is considered a normal response from the point of view of nitrogen balance.

CASE #2:

Clinical History: Patient's general health was excellent until December, 1942. One month previously he had taken a job as a sifter of powder used for fluorescent lights. The atmosphere that the patient worked in was dusty and he was required to wear a mask a greater part of the time while at work. However, at Christmas time in 1942, while walking up a slight incline, he had sudden onset of dyspnea and cough. Although these symptoms continued during the next two months, he gradually improved and returned to work asymptomatic at the end of three months.

From March, 1942 to March, 1945, the patient worked at grinding

tungsten with only minimal exposure to dust. In March, 1945 he began to have daily chills and fever up to 105°F, shortness of breath, and severe coughing spells and he was sent to Trudeau Sanitorium at this time where the abovementioned symptoms continued. He began to note a generalized malaise, anorexia and weight loss. The dyspnea was variable in its intensity, occurring, at times, on the slightest exertion. Paroxysmal dyspnea was also noted and the patient was finally forced to sleep on two pillows at night. The paroxysms of coughing occurred mainly in the morning and at night. The cough was productive of about 1 cupful of watery sputum every day which was occasionally blood-streaked. According to the patient the chills and fever occurred daily for 1½ years. Finally in October, 1946 the patient left Trudeau, having lost 39 pounds, and feeling that his respiratory symptoms were only slightly improved.

He has been under the care of his family doctor since October, 1946 and has been treated with neoarsphenamine, once weekly. He has not been febrile since then and feels that he has been able to tolerate more exertion under this treatment. He has, however, regained only about 7 pounds, during the past year and a half. In addition to the above symptoms, the patient has also noted during the present illness: (1) Frequent muscle cramps in the legs and feet; (2) Frontal throbbing headaches, especially after exertion; (3) Occasional attacks of hunger, sweating and tremor, occurring late in the morning.

Past History and Illnesses: Non-contributory.

Physical Examination: Blood pressure 105/70. P 92. R 24. A well-developed but poorly nourished white male who appeared chronically ill and showed evidence of recent weight loss. At rest, there was apparently no

respiratory distress, though accessory muscles were used. No jaundice. Mild cyanosis and clubbing of fingers. Head and Eyes: negative. Teeth: good. Pharynx: clear. Neck: Thyroid not felt, no venous engorgement. Lymph nodes: one 1 cm. node in the left axilla, soft, non-tender. No other glandular enlargement. Lungs: expansion limited bilaterally, about 2 cm. movement at bases on deep inspiration. Resonance normal. Wet rales are present throughout left lung posteriorly, few in right mid-lung field - do not disappear after cough. Heart: not enlarged to percussion. Rhythm regular. Rate 92. Sounds rather distant, good quality. P_2 accentuated. Abdomen: negative. Extremities: good pulsations, macular rash on calves of legs. Rectal: prostate firm, slightly enlarged.

Laboratory Data:

Wassermann: negative

Blood Count:

RBC 5.5 M

Hgb. 18.5

WBC 6,250

Hct. 42

Differential normal

Urinalysis: negative except for a trace of albumin

Blood Chemistry:

Total proteins 6.9 gm.%

Albumin 5.0 gm.%

Globulin 1.9 gm.%

Blood urea nitrogen 27.2 mg.%

Total bilirubin .53 mg.%

CO_2 53.5 vol.%

Chloride 589 mg.%

Calcium 10.2 mg.%

Cephalin flocculation negative

Thymol turbidity 39

Glucose Tolerance Test: after 50 gm. glucose, p.o.

Fasting 66 mg.%

½ hour 115 mg.%

1 hour 110 mg.%

2 hours 70 mg.%

3 hours 45 mg.%

4 hours 57 mg.%

Insulin tolerance after .1 of a unit of insulin per kg. of body weight, i.v.:

Fasting 66 mg.%

20 minutes 41 mg.%

30 minutes 27 mg.%

45 minutes 45 mg.%

60 minutes 63 mg.%

90 minutes 66 mg.%

120 minutes 70 mg.%

Continuation of Insulin tolerance after .5 cc. of 1:1000 solution of adrenalin hydrochloride:

45 minutes 85 mg.%

60 minutes 66 mg.%

BSP test after 5 mg. of dye per kg.:

15 minutes 100% retention

60 minutes 60% retention

Vitamin A absorption after 7000 I.V. ester Vitamin A per kg. body weight:

Fasting 57 (normal 15-40)

4½ hours 299 (normal about 250)

7 hours 530 (normal about 140)

Electrophoresis Report:

Albumin slightly diminished (3.46 gm.%). Alpha 2 slightly elevated.

Urea Clearance:

74% of normal and 85% of normal.

Urobilinogen in the urine:

.368 Erlich units and .188 Erlich units in 2 hours (2-4 P.M.) Chloride excretion test showed a normal response .238 gm. chloride, as sodium chloride per 100 mls. during the last four hours of the test.

Beryllium Analyses

Case #2

Sample Date	Volume Used	Be Concentration
4/8-4/9/48	200 ml.	--- µg/liter
4/10-4/11	"	---
4/12-4/13	"	---
4/13-4/14	"	0.00
4/14-4/15	"	---
4/16-4/17	"	0.00
4/18-4/19	"	0.06
4/20-4/21	"	0.00

Beryllium Analyses (cont.)

Case #2

Sample Date	Volume Used	Be Concentration
4/22-4/23	200 ml.	0.09
4/24-4/25	"	0.00
4/26-4/27	"	0.02
4/28-4/29	"	0.03
4/30-5/1	"	0.00
5/2-5/3	"	0.00
5/4-5/5	"	0.02
5/10-5/11	"	0.50
5/12-5/13	"	0.05
Date Record Lost	"	0.20
" " "	"	0.04
" " "	"	0.03
" " "	600 ml. (3 samples pooled)	0.18

The samples for which the date was lost belong in the period 4/8 to 4/15/48 .

Samples for which no Be was detected may have a concentration of Be less than 0.01 micrograms per liter.

Respiratory Studies

Although the maximum breath-holding time was but 10 seconds, the maximum breathing capacity was 73 per cent of the predicted value. The total pulmonary lung volume was reduced to almost one-half and the vital

capacity to 39 per cent of the expected value. Bronchospirometry was not done. In addition to sinus tachycardia, the electrocardiogram revealed right axis deviation and peaking of the P waves in lead 2. The venous pressure was increased to 13.5 cm., and the arm to lung circulation time was prolonged to 11 seconds, although the arm to mouth time was within normal limits. Cardiac catheterization showed a moderate increase in right auricular pressure and a marked increase in right auricular pressure and a marked increase in right ventricular pressure compatible with pulmonary hypertension. The cardiac output was abnormally low. The arterial oxygen saturation was diminished to 82 per cent with a pO_2 of 50 mm. Breathing oxygen increased these values to 95 per cent and 114 mm. Pulmonary efficiency in relation to work was markedly impaired, and the maximum incline of grade walking tolerated was 5 per cent. The oxygen debt was abnormally high indicative of circulatory inadequacy. During the period of metabolic study, the possible anabolic effects of testosterone propionate on nitrogen balance were investigated. Patient retained sodium and water, gained weight, exhibited edema together with more severe respiratory distress including dyspnea and orthopnea.

For a week after withdrawal of this medication, symptoms continued, and cardiac catheterization was repeated. The findings were compatible with congestive heart failure, and the initial effects of intravenous digoxin therapy indicated "digitalis effects" in the electrocardiogram. The stroke volume and pulse pressure of the right ventricle promptly improved, but symptoms and signs of pulmonary edema developed because of the abnormal pulmonary resistance to greater blood flow. Phlebotomy promptly alleviated the distress as well as the need for supplemental oxygen therapy.

Patient subsequently diuresed and lost excess weight during the next 24-hour period.

This patient not only exhibited marked impairment of pulmonary diffusion of oxygen, but reduced lung perenchyma. In addition to well-established cor pulmonale, he developed congestive heart failure during a trial of testosterone propionate therapy and responded to intravenous digoxin beyond the capacity of his lungs to accommodate the increased flow of blood.

Metabolic Studies

Other than the procedures listed above, including pulmonary and cardiac studies, metabolism studies were carried out.

The patient was placed on a diet containing 15.28 gms. of nitrogen a day with 2200 calories. Nitrogen balance studies were done during a control period of 6 days followed by 12 days when testosterone propionate was administered (25 mg. q.d., i.m.) and a final control period of 6 days. The nitrogen balance and weight changes during this time are as found below.

Nitrogen Balance

Period*	Diet	Urine	Stool	Balance	Body Weight	Testosterone Propionate mg.
3	15.28	11.26	1.91	+ 2.11	52.37	0
	15.28	12.75	1.91	+ .62		0
	15.28	13.20	1.91	+ .17		0
	15.28	12.0	1.91	+ 1.37		0
	15.28	12.46	1.91	+ .97		0
	15.28	12.80	1.91	+ .47		0
				+ 4.65		

Nitrogen Balance (cont.)

Period*	Diet	Urine	Stool	Balance	Body Weight	Testosterone Propionate mg.
4	15.28	11.80	1.91	+ 1.57	52.06	25
	15.28	11.16	1.91	+ 2.21		25
	15.28	12.03	1.91	+ 1.34		25
	15.28	10.96	1.91	+ 2.41		25
	15.28	11.18	1.91	+ 2.19		25
	15.28	10.35	1.91	+ 3.02		25
				+ 12.74		
5	15.28	10.73	1.91	+ 2.64	53.69	25
	15.28	12.02	1.91	+ 1.35		25
	15.28	11.94	1.91	+ 1.43		25
	15.28	10.48	1.91	+ 2.89		25
	15.28	11.5	1.91	+ 1.87		25
	15.28	12.1	1.91	+ 1.27		25
				+ 11.45		
6	13.98	10.45	1.91	+ 1.62	54.84	0
	13.98	10.25	1.91	+ 1.82		0
	13.98	9.83	1.91	+ 2.22		0
	15.28	12.39	1.91	+ .98		0
	15.98	10.95	1.91	+ 1.12		0
	13.98	10.27	1.91	+ 1.80		0
				+ 9.56		

*Periods 1 and 2 are not reported inasmuch as during this time the subject was approaching nitrogen balance on a control diet and had not reached a constant metabolic state.

The results of these studies might tentatively be interpreted as follows: The patient seemed unable to store nitrogen satisfactorily on an ordinarily adequate protein and caloric intake. The response to testosterone from the point of view of nitrogen storage was minimal, even less than a normal person. However, weight gain was striking and undoubtedly secondary to the storage of electrolytes. The question has arisen as to whether this patient was unable to utilize testosterone propionate because of liver disease. If so, this is of unusual interest because the effect on the electrolyte retention was normal. For some reason this patient was unable to store nitrogen or significantly build up his protein stores even under the powerful anabolic stimulus of testosterone propionate.

The stool nitrogen was high, averaging 1.91 gms. a day. For this reason fat analyses were done on the stool which fell within the normal limits.

CASE #3:

Clinical History: This 35-year old male patient was in good health until March, 1945, when he noted gradual onset of shortness of breath on exertion. Shortly thereafter he began to be irritable and somewhat emotionally unstable. In July, 1945, in addition to the above symptoms, he began to lose weight and lost about 40 pounds in 6 weeks. In August, 1945, he developed a hacking cough. Because of these symptoms he stopped working and sought the help of his family physician. He believed that abnormal markings were found in his x-ray at that time.

During the next 2 years the patient was almost completely incapacitated by his dyspnea and paroxysms of coughing. He was unable to sleep

except in the upright position. Neoarsphenamine was given without benefit.

In July, 1947, penicillin and streptomycin were given by inhalation. His cough became productive and following this the symptoms of persistent pressure on his chest ceased and for the first time he noted improvement of his dyspnea and cough. However, by this time he had begun to use oxygen quite constantly. Although he believed that he has had some relief with each subsequent inhalation therapy of penicillin and streptomycin, he is now, for the most part, bedridden and completely dependent on oxygen. The patient states that he has had mild dependent edema at times during the past year and that clubbing of the fingers has been marked during the past 2 years. There is no history of chills and fever. There has been no loss of appetite even though the weight loss has been marked.

For the past 2 years patient has been passing numerous kidney stones. "I counted up to 50 and stopped." The stones are white or light brown and extremely hard. X-rays are reported to have shown stones on both sides.

From the point of view of occupational history, patient was a foreman in charge of firing beryllium from 1939 to 1942. The atmosphere was not dusty. However, a pungent odor was given off from the furnace during the firing procedure which the patient was aware of. In 1942, he was transferred, where he was plant engineer and has had little exposure to either beryllium dust or fumes since that time.

Past History: Non-contributory.

Physical Examination: Blood pressure 106/60. P 100, R 24. A thin, emaciated white male who appears chronically ill. Some dyspnea at rest. When oxygen was taken away from the patient he became emotionally upset, cyanotic and gasped for breath. Marked clubbing of fingers and toes.

Head: eyes and ears negative. Throat: small tonsils, not injected. Neck: slight venous engorgement, thyroid not felt. Lymph nodes: there is general glandular enlargement, nodes up to 1½ cm., discrete, non-tender. Chest: somewhat barrel-shaped and asymmetrical, left chest being more preminent posteriorly. Lungs: expansion limited. Resonant throughout. Expiration was prolonged and occasionally expiratory wheezes were heard. Moist rales which did not disappear with cough were present over lower half of right chest. Heart: not enlarged to percussion. RSR. First sound split at apex. Soft systolic murmur at apex. P_2 was greater than A_2. Abdomen: scaphoid. Liver and spleen not felt. Right kidney palpable, probably slightly enlarged, tender. Extremities: negative except for the clubbing, no edema. Rectal: prostate tender, not enlarged.

Admission Laboratory Data:

Blood Count:

RBC 3.42 M.

Hgb. 11.5 gms.

WBC 15,250

Hct. 38.1

Platelets adequate

Urinalysis:

Specific Gravity 1.008

Albumin 2+

Microscopic loaded with red blood cells and white blood cells.

Wassermann: negative

Blood Chemistry:

Total proteins 6.9 gms.%

Albumin 3.2 gms.%

Globulin 3.7 gms.%

Blood Urea Nitrogen 16.6 mg.%

CO_2 77 volumes%

Chloride 88 meq.

Calcium 9.6 mg.%

Phosphorous 2.6 mg.%

Cephalin flocculation 2+

Thymol turbidity 53

Bilirubin total .174 mg.%

Glucose tolerance test after 50 gm. glucose, p.o.:

Fasting 46 mg.%

½ hour 115 mg.%

1 hour 130 mg.%

2 hours 108 mg.%

3 hours 93 mg.%

Insulin tolerance test after .1 of a unit of insulin per kg. of body weight, i.v.:

Fasting 64 mg.%

20 minutes 57 mg.%

30 minutes 42 mg.%

45 minutes 42 mg.%

60 minutes 57 mg.%

90 minutes 50 mg.%

120 minutes 64 mg.%

Continuation of insulin tolerance after .5 cc. of 1:1000 solution of adrenalin hydrochloride:

45 minutes 71 mg.%

60 minutes 64 mg.%

Vitamin A absorption after 7000 I.V. ester Vitamin A per kg. body weight:

Fasting 37 Evelyn Units 37 (normal 15-40)

4½ hours 432 (normal about 250)

7 hours 312 (normal about 140)

BSP test after 5 mg. of dye per kg.:

15 minutes 40% retention

45 minutes 20% retention

PSP Test:

Less than 5% in 15 minutes

55% in 2 hours

Cutler Wilder Test:

.0998 gms. chloride as sodium chloride in 100 ml.

Electrophoresis report:

Albumin 2.7%

α 1 0.47

α 2 1.01

β 1.07

ϕ 1.07

γ 1.34

Interpretation: Pattern very abnormal. is the only constituent in normal concentration. Albumin is markedly diminished. α 2, β, ϕ, γ are increased, especially the last two.

X-rays: Other than those included in the cardio pulmonary studies were KUB, I.V. Pyelogram and x-rays of the long bones. KUB and I.V. Pyelogram showed many calcific densities in the region of both kidneys. Apparently each group of calyces has one or more stones in its vicinity. X-rays of the long bones showed no decalcification.

Course in Hospital: During the hospital stay, the patient was afebrile, but ran a persistent tachycardia in the neighborhood of 100 per minute. He required continuous oxygen and his stymptoms of dyspnea and cough remained quite constant throughout the period of investigation.

Beryllium Analyses

Case #3

Sample Date	Volume Used	Be Concentration
5/8-5/9/48	200 ml.	0.00 µg/liter
5/10-5/11	"	0.02
5/12-5/13	"	0.00
5/14-5/15, 5/16-5/17, 5/18-5/19	pooled 600 ml.	0.025
5/20-5/21	200 ml.	0.00
5/22-5/23	"	0.00
5/24-5/25	"	0.02
5/26-5/27, 5/28-5/29, 5/30-5/31	pooled 600 ml.	0.020

Respiratory Studies

The maximum breathing capacity was greatly reduced to 15 per cent of the predicted value. Breath-holding time varied between 5 and 17 seconds. The alveolar pressure - velocity relationships were borderline normal. The total pulmonary lung volume was reduced to one-half the predicted value, and the vital capacity to 40 per cent. Bronchospirometry was not attempted. The electrocardiogram showed right axis deviation and right heart strain with inversion of T waves in leads 2 and 3 (while taking digitalis). The circulation times were normal, but the peripheral venous pressure, despite gross venous distension, was repeatedly observed to vary between minus 1 and 2 c water. The systemic blood pressure was low. Cardiac catheterization showed the right auricular and ventricular pressures above normal, and there was definitely pulmonary hypertension. While breathing oxygen the cardiac output was normal, and the arterial oxygen saturation was 89 per cent with a pO_2 of 95 mm. Removal of the supplemental oxygen promptly lowered the saturation within seconds, and the pO_2 fell to 46 mm. while breathing room air. Because of this patient's marked respiratory disability, no studies of exercise tolerance could be done.

For investigational purposes, the patient was catheterized a second time and the initial effects of cytochrome C injected intravenously were observed. The changes indicative of greater tissue utilization of oxygen claimed by Proger et al, and denied by various other investigators, were not observed.

This patient presented the most severe respiratory impairment of any patients in this group that have been studied to date. Because of the marked impairment of oxygen diffusion resulting in severe arterial anoxemia,

he needs the continuous supply of oxygen which he demands. Despite the evidence of pulmonary hypertension and cor pulmonale, there is no evidence of congestive heart failure and the withdrawal of digitalis as well as the later administration of digoxin were found to make no significant difference.

He was placed on a diet containing 2900 calories and 8.3 gms. of nitrogen. The nitrogen balance during 12 days on this diet is reported below and on the following page. Following this, the patient complained of persistent hunger and his diet was increased to 3300 calories and contained 19.3 gms. of nitrogen per day. The balance during the following 12 days is also tabulated.

Nitrogen Balance

Period	Diet	Urine	Stool	Balance	Urine Chloride	Body Weight
1	18.3	13.65	2.34	+ 2.31		51.89
	18.3	14.83	2.34	+ 1.13		
	18.3	14.2	2.34	+ 1.76		
	18.3	13.39	2.34	+ 2.57		
	18.3	13.9	2.34	+ 2.06		
	18.3	14.78	2.34	+ 1.18		
				+11.01		
2	18.3	14.6	2.34	+ 1.36	7.23	51.68
	18.3	14.80	2.34	+ 1.16	7.79	
	18.3	14.53	2.34	+ 1.23	6.20	
	18.3	15.53	2.34	+ .43	6.90	
	18.3	16.18	2.34	- .22	6.90	
	18.3	14.14	2.34	+ 1.82	7.40	
				+ 5.76	42.40	

Nitrogen Balance (cont.)

Period	Diet	Urine	Stool	Balance	Urine Chloride	Body Weight
3	19.3	14.1	2.34	+ 2.86	9.7	53.27
	19.3	14.42	2.34	+ 2.54	7.8	
	19.3	14.52	2.34	+ 2.44	8.9	
	19.3	14.38	2.34	+ 2.58	9.9	
	19.3	16.28	2.34	+ .68	10.6	
	19.3	16.13	2.34	+ .83	9.6	
				+11.93	56.5	
4	19.3	14.6	2.34	+ 2.36	11.20	54.26
	19.3	15.9	2.34	+ 1.06	11.62	
	19.3	14.88	2.34	+ 2.08	8.60	
	19.3	13.8	2.34	+ 3.16	8.50	
	19.3	13.37	2.34	+ 3.59	9.20	
	19.3	13.44	2.34	+ 3.52	9.80	
				+15.77	58.92	

Interpretation: The results show that this patient gained some weight during the 24-day experimental period, (about 2.2 kg.), and that he stored approximately 24 gms. of nitrogen during this time. However, it was felt that his response was probably not as great as that of other patients, similarly malnourished and on equivalent dietary intake. In the study of such cases as much as 3-4 gms. of nitrogen have been stored per day. The irregularity of the weight gain in this case, we believe, was due to the changes in the water and electrolyte content of the body. Also to be noted in this patient is the excessively high stool nitrogen averaging 2.34 gm. of nitrogen daily in the stool.

Summary: Three additional patients showing sighs of chronic pulmonary granulomatosis of beryllium workers have been studied. Significant data as determined from detailed case histories, clinical and laboratory examinations are given; positive results gained from thorough study of respiratory physiology are given; collected data from the study of the nitrogen metabolism on controlled dietary regime are included.

Significant findings in the above studies can be listed as follows:

1. All of the patients studied showed clinical and radiological evidence of the presence of the disease.

2. Only the advanced case showed any evidence of hematological abnormality in the presence of a secondary anemia.

3. The moderately advanced and advanced cases showed evidence of functional liver abnormality in the presence of abnormal bromsulphalein retention. The Vitamin A absorption tests give presumptive evidence of liver abnormality but the true significance of this test is as yet undetermined. Only in the advanced case (#3) were disturbances in the albumin-globulin ratio, cephalin flocculation and thymol turbidity noted.

4. Spectrographic determinations of 24-hour urine specimens showed the intermittent detection of the substance in small amounts in all three patients. No conclusions can be drawn from this observation at the present time inasmuch as the frequency of the presence of beryllium in normal urine has not been studied.

5. Summarization of the observed findings of respiratory nature reveals:

a. a reduction in maximum breathing capacity and total pulmonary capacity in the moderately advanced and advanced cases.

b. reduction in vital capacity in all cases.

c. electrocardiographic observation of right axis deviation in the presence of evidence of an elevated right ventricular pressure and a cor pulmonale.

d. prolongation of the arm to lung circulation times in the presence of a normal arm to mouth circulation time.

e. a diminution of the arterial oxygen saturation to 82-85 per cent in the two cases not requiring oxygen with a pO_2 of 50-75 mm.

f. pulmonary efficiency calculated in relation to work (for method see last quarterly report, Rochester Report No. UR-21) below normal in all cases.

6. Nitrogen balance studies on two of the three patients (Cases #2 and #3) showed:

a. a high fecal nitrogen, in the absence of diarrhea without a corresponding increase in the excretion of fat (determined only in Case #2).

b. poor utilization of the nitrogen which is absorbed.

The mechanism of these two factors remains unknown. Factor #1 could be due to poor absorption of protein or possibly to reexcretion of nitrogenous products into the gut.

Because of what appears to be the normal response in Case #1, one assumes that disturbance in nitrogen metabolism occurs only in well-advanced cases. However, it must be stressed that no definite conclusion can be drawn from three cases and it is recommended that nitrogen, sodium

and chloride balance be carried out on several more patients. Probably calcium and phosphorous balances should also be done on at least one patient to help substantiate the poor utilization of food protein for building body tissue.

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