LOS ALAMOS SCIENTIFIC LABORA
OF THE [illegible] LOS ALAMOS, NEW ME

[illegible] RESEARCH GROUP (H-4)

[illegible] SEMIANNUAL REPORT

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LOS ALAMOS SCIENTIFIC LABORATORY
OF THE UNIVERSITY OF CALIFORNIA LOS ALAMOS NEW MEXICO

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BIOLOGICAL AND MEDICAL RESEARCH GROUP (H-4) OF THE HEALTH DIVISION - SEMIANNUAL REPORT JULY THROUGH DECEMBER 1959

Group Leader, W. Langham
Division Leader, T. L. Shipman

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CHAPTER 1

INTRODUCTION

The Biological and Medical Research Group (Group H-4 of the Health Division) of the Los Alamos Scientific Laboratory has not issued progress reports since 1957. Rather the policy has been to report work only when complete in the form of LA-documents or as manuscripts submitted directly to national and international scientific journals. Although publication directly in the periodical literature probably results in the widest distribution of the group's scientific contributions, it does not provide the Laboratory Director, the AEC's Division of Biology and Medicine, other national laboratories, and other interested agencies with an easily accessible and continuing account of the group's over-all research effort and progress. A number of members of the staff of the Division of Biology and Medicine have expressed their desire for a periodic progress reporting system to aid them in program planning and budgeting operations. Prior to

1957, H-4 issued abstracts of completed projects and work in progress, either separately or as part of the Health Division's annual report. Short abstracts were included also in the Health Division's monthly progress reports to the Laboratory Director. It seems now that both of these systems of reporting failed to satisfy the needs of all persons and agencies interested in following the programs of the AEC's national laboratories.

This document is a first attempt at semiannual reporting to see if it provides a more satisfactory means of disseminating information on the group's program. This system of reporting is based largely on that of the Argonne National Laboratory's Divisions of Biological and Medical Research, and Radiological Physics -- modified to fit the standard format adopted for Los Alamos Scientific Laboratory reports.

The Biological and Medical Research Group is divided into the following sections: Biochemistry, Low-level Counting, Organic Chemistry, Radiobiology, Radiopathology, and Veterinary Services. Reports of the various projects of each section constitute a chapter of the general report. The project reports are, in most cases, documentation of progress made during the past six months and do not constitute finished manuscripts or final interpretations, and frequently data and conclusions are to be considered as interim or preliminary in nature. At the

end of each chapter is a title list of reports and articles written by section members during the report period. These articles represent projects finished and published in the open literature, accepted for publication, or distributed as LA-documents.

The personnel of the group as of December 31, 1959, their qualifications, classification, and group and section affiliation are shown by the following table of organization.

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CHAPTER 2

BIOCHEMISTRY SECTION

Conjugation of Taurine and Cholic Acid in Irradiated Animals

(D. F. Petersen and R. G. Gould)

Introduction

Enhanced urinary excretion of taurine by irradiated animals (1,2) and man (3,4) appears to be limited quantitatively by a mechanism involving either selective oxidation of a sulfhydryl precursor, or the liberation of finite amounts of preformed taurine (5,6). Participation of the pancreas in the production of excessive amounts of taurine (6) suggested that the target organ involved in conversion of precursors to free taurine might well be the liver and that failure of the conjugation of taurine with cholic acid might explain renal, rather than biliary, clearance.

Experimental Procedure

Taurine conjugation by liver homogenates derived from

normal and irradiated (600 rads) rats was measured by the method of Bremer (7) employing S^{35}-labeled taurine. Direct measurement of in vivo conversion of taurine to taurocholic acid was accomplished by determining free and conjugated S^{35}-taurine in the urine and bile of control and irradiated bile fistula rats. Total taurine was measured by the method of Ling (8).

Results and Discussion

Measurements of taurocholate conjugation by normal and irradiated rat liver homogenates are summarized in Table 1. These data indicate that after doses ranging from 100 to 1600 rads of whole body X irradiation the most pronounced effect on conjugation occurred following a dose of 200 rads. Doses of this size were previously shown to cause taurine production in the greatest amount (5). Thus, these data demonstrate that the conjugation mechanism in the liver is operative and that the taurine produced can be conjugated. Since cholate was present in excess (7), these results are interesting in that conjugation capacity followed taurine production rather than decreasing it, as would be expected if the hypothesis proved to be correct. At higher doses of radiation, the extent of conjugation was essentially the same as that noted in the control animals. At the present stage

TABLE 1. CONJUGATION OF S^{35}-TAURINE WITH CHOLIC ACID BY LIVER HOMOGENATES*

Animals (No.)	Radiation Dose (rads)	Time of Sacrifice (hours)	S^{35}-Taurine Added (c/min)	S^{35}-Taurine Recovered as Taurocholate (c/min)	Conjugation (%)
1	Control	5	25,109	1737	6.9
2	Control	5	25,109	3001	12.0
3	100	5	25,109	2118	8.7
4	100	5	25,109	2992	11.9
5	200	5	25,109	5508	21.9
6	200	5	25,109	5297	21.1
7	400	5	25,109	3100	12.3
8	400	5	25,109	3710	14.8
9	800	5	25,109	2687	10.7
10	800	5	25,109	1772	7.1
11	1600	5	25,109	2041	8.1
12	1600	5	25,109	1755	7.0

*Flask additions: 2 ml 25 per cent liver homogenate in Bucher medium and 0.4 ml S^{35}-taurine = 25,109 c/min; 0.2 ml 0.01 ATP. Total volume: 2.6 ml.

of development, these data are difficult to interpret in the absence of information regarding the total taurine and cholate content of the liver after various doses of radiation. Experiments are currently in progress to clarify this point and should indicate whether the amount of taurine present is the limiting factor involved in conjugation, or whether after doses in excess of 200 rads conjugation actually fails.

A second experiment was performed to test directly whether excessive taurine excretion arises from preformed taurine or from other sulfhydryl precursors. Four μc of S^{35}-taurine was injected into bile fistula rats, and excretion of the S^{35}-label was followed both in the bile and the urine. These data (summarized in Table 2) indicated that during the first 21 hours after 600 rads, taurine excretion by both renal and biliary routes was essentially the same as that observed in nonirradiated controls. Total taurine was determined and specific activities calculated for the first six hours of excretion. These analyses failed to show significant changes due to radiation exposure. Thus, these data confirm the observation (9) that enhanced taurine excretion due to radiation exposure does not arise from preformed taurine. It is anticipated that experiments currently in progress employing S^{35}-sulfhydryl compounds may define further the metabolic

TABLE 2. CUMULATIVE RENAL AND BILIARY EXCRETION OF S^{35}-TAURINE BY IRRADIATED BILE FISTULA RATS*

Sampling Intervals (hours)	Urine				Bile			
	Controls		600 rads		Controls		600 rads	
	c/m x 10^{-3}	% Dose	c/m x 10^{-3}	% Dose	c/m x 10^{-3}	% Dose	c/m x 10^{-3}	% Dose
3	190	10.0	123	6.5	31.5	1.7	40.5	2.1
6	283	14.9	197	10.4	77.0	4.1	70.9	3.7
12	391	20.6	273	14.4	151.7	8.0	136.8	7.2
21	441	23.2	347	18.3	215.7	11.4	193.4	10.2
Specific Activity (0 to 6 hour pooled samples c/m/μM taurine)	32,720		29,211		5,287		4,473	

*S^{35}-taurine (1.8×10^6 c/m) administered intraperitoneally immediately prior to collection. Animals were irradiated 30 minutes before labeling.

pathway leading to radiation-induced excessive taurine excretion.

REFERENCES

(1) R. E. Kay and C. Entenman, Fed. Proc. 13, 520 (1954).

(2) R. B. Mefford and H. H. Mortens, Science 122, 829 (1955).

(3) A. L. Kretchmar, Oak Ridge Institute of Nuclear Studies Report ORINS-25 (M. Brucer, ed.), p. 4.1 (1959).

(4) D. F. Petersen and C. C. Lushbaugh, submitted to J. Occup. Med.

(5) R. E. Kay, J. C. Early, and C. Entenman, Rad. Res. 6, 98 (1957).

(6) R. E. Kay and C. Entenman, Rad. Res. 11, 357 (1959).

(7) J. Bremer, Acta Chem. Scand. 9, 683 (1955).

(8) N. R. Ling, J. Clin. Path. 10, 100 (1957).

(9) D. N. Stern and E. M. Stim, Proc. Soc. Exptl. Biol. Med. 101, 125 (1959).

Serum Enzymes after X-ray and Gamma-Neutron Irradiation

(D. F. Petersen and L. B. Cole)

Introduction

Previous studies in this laboratory (1,2) and by other investigators (3,4) have shown that activity of a number of enzymes normally found in the serum is increased by exposure to ionizing radiations. While some disagreement exists regarding the time of onset, magnitude, and duration of radiation induced increases in serum enzyme content, it has been generally accepted that owing to variability of the response of common laboratory species serum glutamic-oxalacetic transaminase (GOT) measurements in particular are unsatisfactory as an index of radiation injury. The present study was undertaken to assess factors responsible for the variability and to determine the fate of enzymes released by injured tissues into the peripheral circulation.

Experimental

Adult male Sprague-Dawley rats, female New Zealand rabbits, and male rhesus monkeys (Macaca mulatta) were used for these experiments. Rats and rabbits were given 250 KVP X irradiation, and the monkeys were exposed to the degraded fission spectrum (23 per cent gamma contaminant) of the

Godiva II assembly. Glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), lactic dehydrogenase (LDH), and malic dehydrogenase (MDH) were assayed by modifications of existing methods (5-8) based on spectrophotometric measurement of the rate of disappearance of reduced diphosphopyridine nucleotide at 37.5°C. Activity was expressed in terms of μM of product formed per ml of serum per hour. Bile fistula rats were prepared by catheterization of the common duct with No. 10 polyethylene tubing under pentobarbital anesthesia. The animals were subsequently maintained in Bollman cages (9) and given 5 per cent dextrose in n-saline in place of tap water. Carbon tetrachloride (CCl_4, 795 mg/kg) in corn oil was injected subcutaneously or by stomach tube as indicated in Table 1.

Results and Discussion

Comparison of the enzyme content of serum samples drawn immediately before and 30 minutes after exposure of rhesus monkeys to doses of gamma-neutron irradiation ranging from 187 to 388 rads is shown in Table 1. Increases in serum enzyme activity ranging to 246 per cent of the corresponding preirradiation value were found, but no consistent relationship between dose and the magnitude of the response was observed. It appeared, therefore, that within the range of

TABLE 1. Influence of Gamma-Neutron Irradiation on Serum Enzyme Concentrations in the Monkey

Group	Dose (rads)	Mean Activity (μM/ml/hr)	Standard Error	Preirradiation Value (%)
Glutamic-oxalacetic Transaminase (GOT)				
Control	0	1.18	0.051	100
Group I	195	1.00	0.078	85
Group II	271	1.94	0.076	164
Group III	287	1.38	0.098	117
Group IV	337	1.78	0.116	151
Group V	388	1.62	0.137	137
Glutamic-pyruvic Transaminase (GPT)				
Control	0	0.862	0.046	100
Group I	195	0.916	0.079	106
Group II	271	1.200	0.071	139
Group III	287	0.938	0.068	109
Group IV	337	1.040	0.051	121
Group V	388	0.683	0.037	79
Lactic Dehydrogenase (LDH)				
Control	0	23.9	1.00	100
Group I	195	20.9	2.53	87
Group II	271	53.1	5.29	222
Group III	287	31.8	0.48	133
Group IV	337	51.1	3.84	214
Group V	388	26.4	2.66	110
Malic Dehydrogenase (MDH)				
Control	0	11.3	0.057	100
Group I	195	27.9	--	246
Group II	271	24.9	1.92	220
Group III	287	18.2	2.50	161
Group IV	337	21.2	2.00	187
Group V	388	15.6	1.79	138

radiation doses where such estimations would be most useful, quantitative evaluation of the injury sustained could not be made. In this respect, the data are similar to results previously obtained from cancer patients (1) and from a recent criticality accident victim (10).

Since the amount of injury caused by a given dose of radiation must be relatively constant, the variability observed in animal populations suggested that a physiologic mechanism not particularly sensitive to ionizing radiations prevented accumulation of large amounts of enzyme in the circulation. This idea was tested by measuring the rate of disappearance of GOT activity after intravenous administration of homologous myocardial enzyme to irradiated and non-irradiated rabbits. Data shown in Fig. 1 indicated that LD_{50} doses of radiation did not influence the rate of disappearance of circulating enzyme. Dilution of known amounts of injected enzyme activity was consistent with the idea (11) that GOT activity is in equilibrium with the total extracellular fluid compartment. Thus 5 cc of an enzyme preparation containing 2.3×10^3 μM/ml/hr increased serum GOT levels 10 to 15 fold, and rapid disappearance indicated the large capacity of the inactivation system.

The possibility that the liver played an important role in inactivation of circulating serum enzymes was investigated

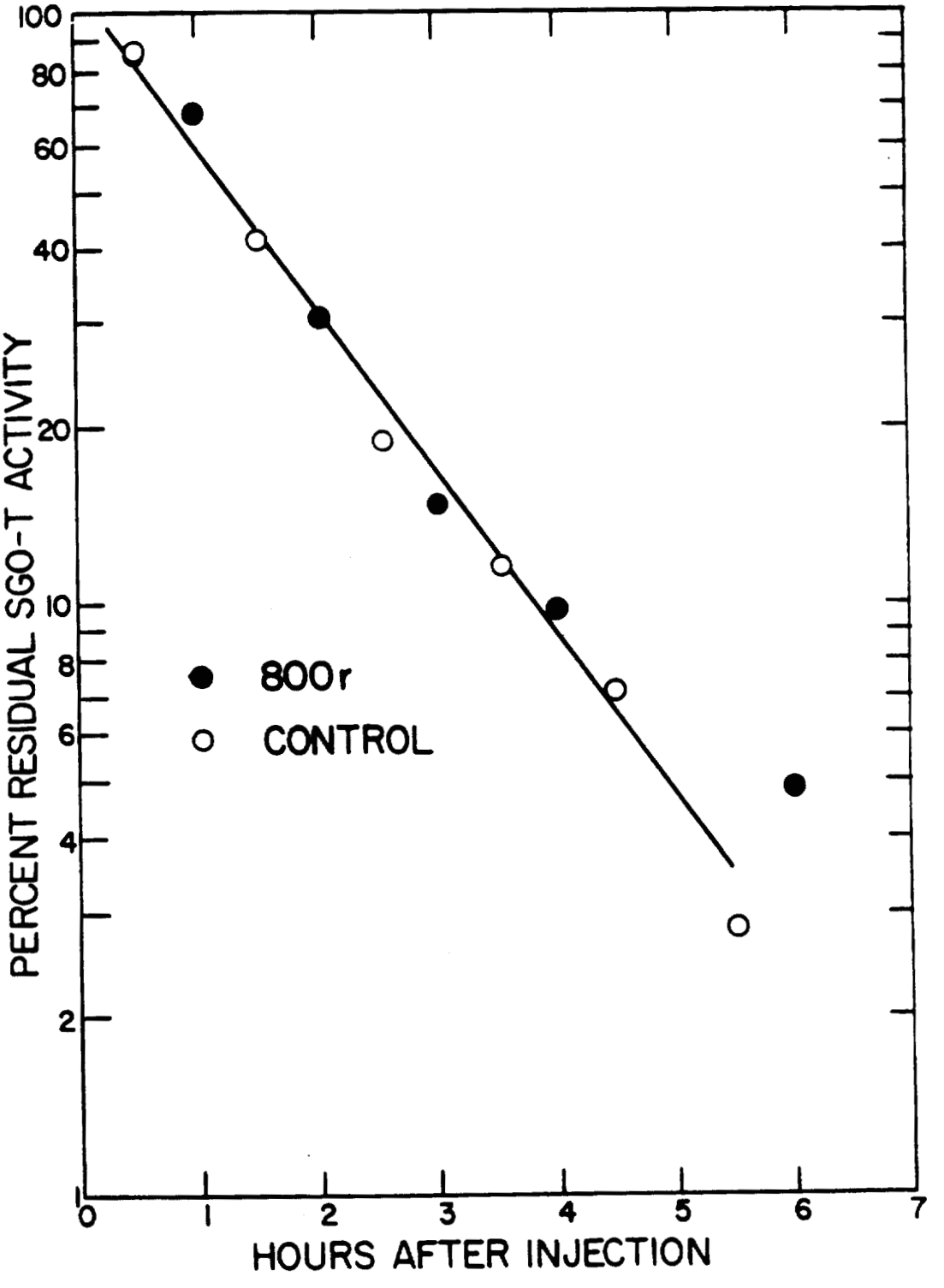


Fig. 1. Disappearance of injected GOT activity from the peripheral circulation.

by causing liver damage and subsequently measuring the net increase in serum enzyme accumulation due to irradiation, in comparison with CCl_4-poisoned controls. These results, summarized in Table 2, demonstrated that animals with impaired hepatic function generally exhibited much greater net increases in serum enzyme accumulation due to irradiation than nonpoisoned, irradiated animals.

In order to approach directly the question of enzyme inactivation by biliary excretion, bile fistula animals were irradiated (800 rads) and GOT was measured frequently in the collected bile. Normal rat bile contains approximately half the GOT activity per unit volume of rat serum, in contrast to human gall bladder bile which has much higher activity (12). Figure 2 shows the effect of 800 rads on biliary excretion of GOT activity. These data indicated that enhanced GOT activity appeared briefly during the first 4 hours after irradiation and rapidly returned to normal levels. More persistent excretion was observed in CCl_4-poisoned animals, but we cannot as yet rule out the possibility that the GOT activity appearing in bile was of hepatic origin. However, based on the assumption that GOT activity in the bile was of circulatory origin and in view of the dilution of injected enzyme activity described above, biliary excretion could not account for the disappearance

TABLE 2. Influence of Hepatic Injury on Accumulation of Serum Enzymes in Irradiated Rats

Group	0 hrs	3 hrs			6 hrs		
	(μM/ml/hr)	(μM/ml/hr)	Control (%)	Net Increase (%)	(μM/ml/hr)	Control (%)	Net Increase (%)
Glutamic-oxalacetic Transaminase (GOT)							
Control	6.8	6.8	100	-	10.3	151	-
CCl_4		9.1	133	-	12.2	180	-
CCl_4 + 800 rads		14.8	218	185	21.9	322	194
800 rads		8.8	130	129	12.0	176	116
Glutamic-pyruvic Transaminase (GPT)							
Control	1.7	1.6	95	-	1.4	83	-
CCl_4		2.1	125	-	2.9	171	-
CCl_4 + 800 rads		5.3	313	300	3.5	206	143
800 rads		2.4	141	149	2.0	118	143
Lactic Dehydrogenase (LDH)							
Control	60.6	82.5	136	-	103.0	170	-
CCl_4		134.8	222	-	86.0	142	-
CCl_4 + 800 rads		167.0	275	139	240.0	396	233
800 rads		107.0	176	130	117.7	194	114
Malic Dehydrogenase (MDH)							
Control	22.6	22.6	100	-	33.5	148	-
CCl_4		66.8	295	-	58.9	260	-
CCl_4 + 800 rads		154.0	680	486	474.0	2095	1340
800 rads		41.1	182	182	45.2	200	135

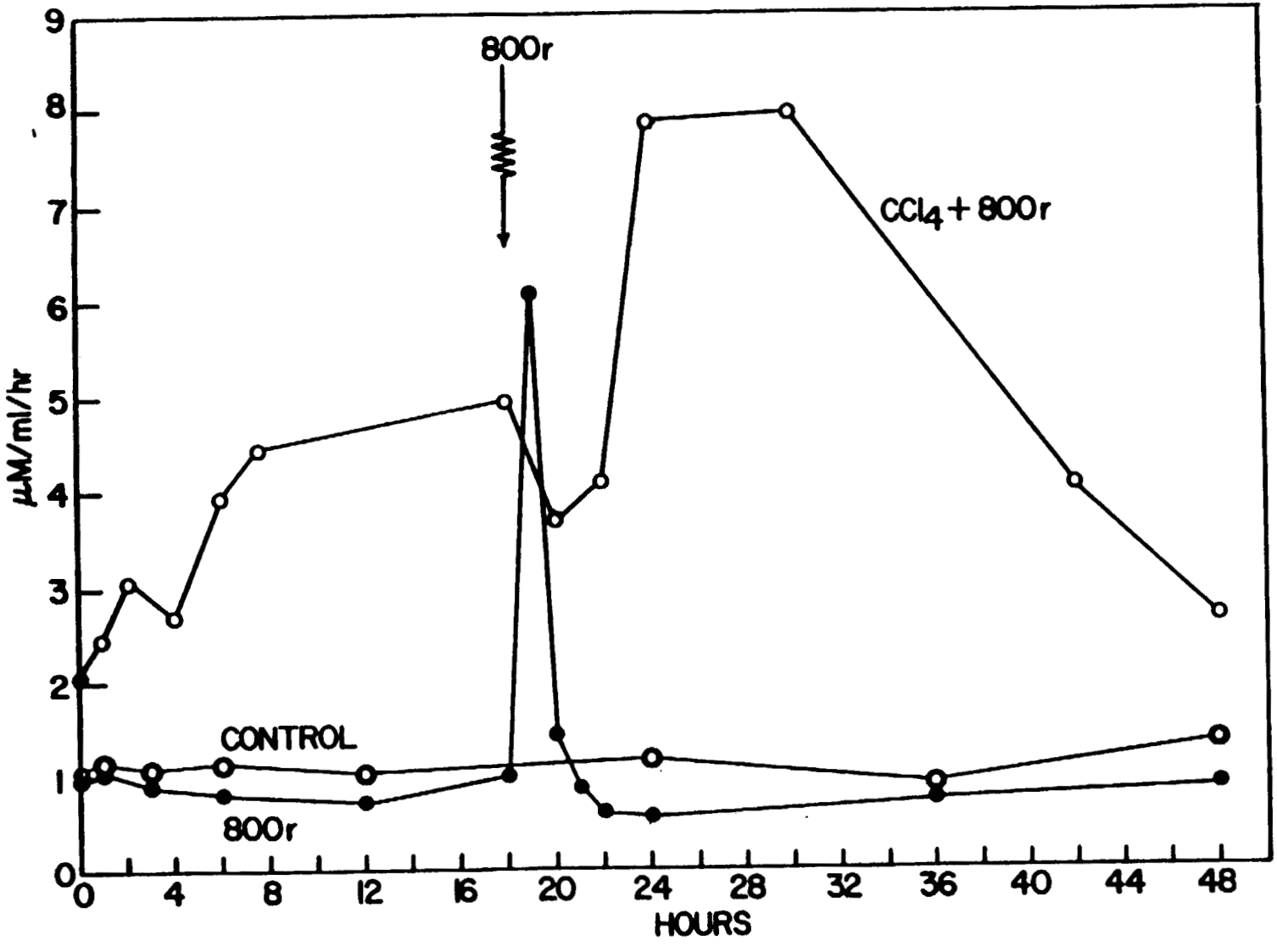


Fig. 2. Biliary excretion of glutamic-oxalacetic transaminase activity.

of more than ~5 per cent of the total enzyme inactivated. These results are in agreement with data previously reported by Dunn and co-workers (11) and show conclusively that biliary excretion is not a significant avenue for elimination of serum enzyme activity. In the absence of appreciable urinary or biliary excretion (2) and in view of the marked effect of hepatotoxins on radiation induced accumulation of serum enzymes, it seems reasonable to postulate an inactivation mechanism in the hepatic parenchyma. Resistance to radiation, together with the remarkable capacity of the mechanism to inactivate circulating enzyme activity, appears to explain the failure of serum GOT measurements as a suitable indicator of radiation injury.

REFERENCES

(1) D. F. Petersen, C. C. Lushbaugh, and P. Lee, Fed. Proc. 16, 327 (1957).

(2) D. F. Petersen and L. B. Hughes, Rad. Res. 9, 166 (1958).

(3) L. J. Milch and H. G. Albaum, Proc. Soc. Exptl. Biol. Med. 93, 595 (1956).

(4) G. Kessler, M. B. Hermel, and J. Gershon-Cohen, Proc. Soc. Exptl. Biol. Med. 98, 201 (1958).

(5) A. Karmen, J. Clin. Invest. 34, 131 (1955).

(6) F. Wroblewski and J. S. LaDue, Proc. Soc. Exptl. Biol. Med. 91, 569 (1956).

(7) B. R. Hill and C. Levi, Cancer Res. 14, 513 (1954).

(8) A. Siegel and R. J. Bing, Proc. Soc. Exptl. Biol. Med. 91, 604 (1956).

(9) J. L. Bollman, J. Lab. Clin. Med. 33, 1348 (1948).

(10) D. F. Petersen and C. C. Lushbaugh, submitted to J. Occup. Med.

(11) M. Dunn, J. Martins, and K. R. Reismann, J. Lab. Clin. Med. 51, 259 (1958).

(12) M. Chinsky, G. L. Shmagranoff, and S. Sherry, J. Lab. Clin. Med. 47, 108 (1956).

Nuclear Phosphorylation in Hematopoietic Tissues (D. F. Petersen and L. B. Cole)

Introduction

Creasy and Stocken (1,2) have recently reported that nuclear phosphorylation of bound nucleotides, originally described in calf thymus by Osawa, et al (3), is a peculiar property of radio-sensitive tissues of the rat. The process appears to be remarkably radio-sensitive both in vivo and in vitro. Inhibition was detectable almost immediately after 25 rads, 80 to 100 per cent complete after 100 rads, and reversible in vivo within 4 to 5 days. Ord and Stocken (4) have suggested that the radiosensitivity of various cell types thus may be related to the contribution of nuclear reactions to the cellular economy and the capacity of cytoplasmic reactions to reverse radiation induced nuclear disorders. This report summarizes results of experiments designed to assess the nuclear-cytoplasmic interactions affecting nucleotide polyphosphate concentrations in the spleen and thymus during the early post irradiation period.

Experimental

Young adult male Sprague-Dawley rats weighing 180 to 200 g were used to make these studies. Nuclei were prepared in sucrose-$CaCl_2$ by a modification of the method described by

Osawa (3) and in the buffered medium of Creasy and Stocken (2). Following incubation, nucleotides in the acid-soluble fraction were adsorbed on Norit and hydrolyzed by the method of Crane and Lipmann (5). Orthophosphate liberated during hydrolysis was estimated by the method of Fiske and Subbarow (6). Incorporation of P^{32} into nuclear nucleotide polyphosphates was accomplished by isolation of nuclei in sucrose-$CaCl_2$ medium containing 10^{-3} M NaCN, 20 minutes after exposure to 400 rads and injection of 100 μc P^{32}-orthophosphate. Nucleotides in the trichloroacetic acid (TCA) extract of nuclei were applied to Whatman No. 1 paper and separated in a modification of the two-step solvent system of Krebs and Hems (7). Spots were located in transmitted ultraviolet light with an intensification screen (8), identified by correspondence with authentic markers developed simultaneously, cut out, and counted with an end window Geiger-Müller tube. Nucleotides were eluted in 0.1 N HCl and estimated by the method of Vischer and Chargaff (9). Deoxyribonucleic acid phosphorus was measured in the trichloroacetic acid precipitate by Schneider's modification (10) of the Dische diphenylamine reaction.

Results and Discussion

The effect of 100 rads of X irradiation on phosphorylation of bound nucleotides of spleen and thymus nuclei is

summarized in Table 1. These data demonstrate that esterification of nucleoside polyphosphate was completely inhibited within 5 minutes after exposure. Results with lower doses of radiation were quite variable in our hands, but in view of the differences in methods, these data are in essential agreement with the results initially reported by Creasy and Stocken (2).

TABLE 1. Nuclear Phosphorylation after Irradiation in vitro

Tissue	Dose (rads)	Time after X ray (minutes)	μgP/mg DNA-P/min
Spleen	Control	5	0.65
Spleen	100	5	0.00
Thymus	Control	5	0.33
Thymus	100	5	0.00

Details of the phosphorylative process in nuclei have not as yet been well defined. However, it has been shown that after isolation, degradation of nuclear nucleoside polyphosphates occurs as a function of time in the absence of oxygen and that phosphorylation occurs when oxygen is restored to the system (3). Since degradation occurs slowly at 0°C, it was reasoned that by including cyanide (3) in the

isolation medium and working rapidly in the cold, the nucleotide complement present in the nucleus at the time of sacrifice could be determined.

Figure 1 shows the concentration of adenine nucleotides in the spleen at intervals of 10, 20, and 30 minutes after exposure to 200 rads. Contrary to what might be expected on the basis of the in vitro experiments, the total nuclear adenine nucleotide content of the spleen decreased during the first 10 minutes after irradiation and subsequently increased to greater than control values at 30 minutes. Furthermore, the accumulation consisted not of adenylic monophosphate but rather resulted from a disproportionate increase in adenosine diphosphate and adenosine triphosphate.

Figure 2 demonstrates that a similar situation existed in the thymus gland. These observations are difficult to reconcil with the previous view of the significance of radiation effects on nuclear nucleotide polyphosphates (4) and indicate rather that the nuclear defect in vivo may be related to the transfer of phosphate energy to deoxynucleoside monophosphates. However, recent studies by Kier and Davidson (11) have questioned the validity of nucleotide measurements conducted on material isolated in aqueous media, and further studies are necessary to establish whether nucleotides estimated by this method represent the actual nuclear nucleotide content.

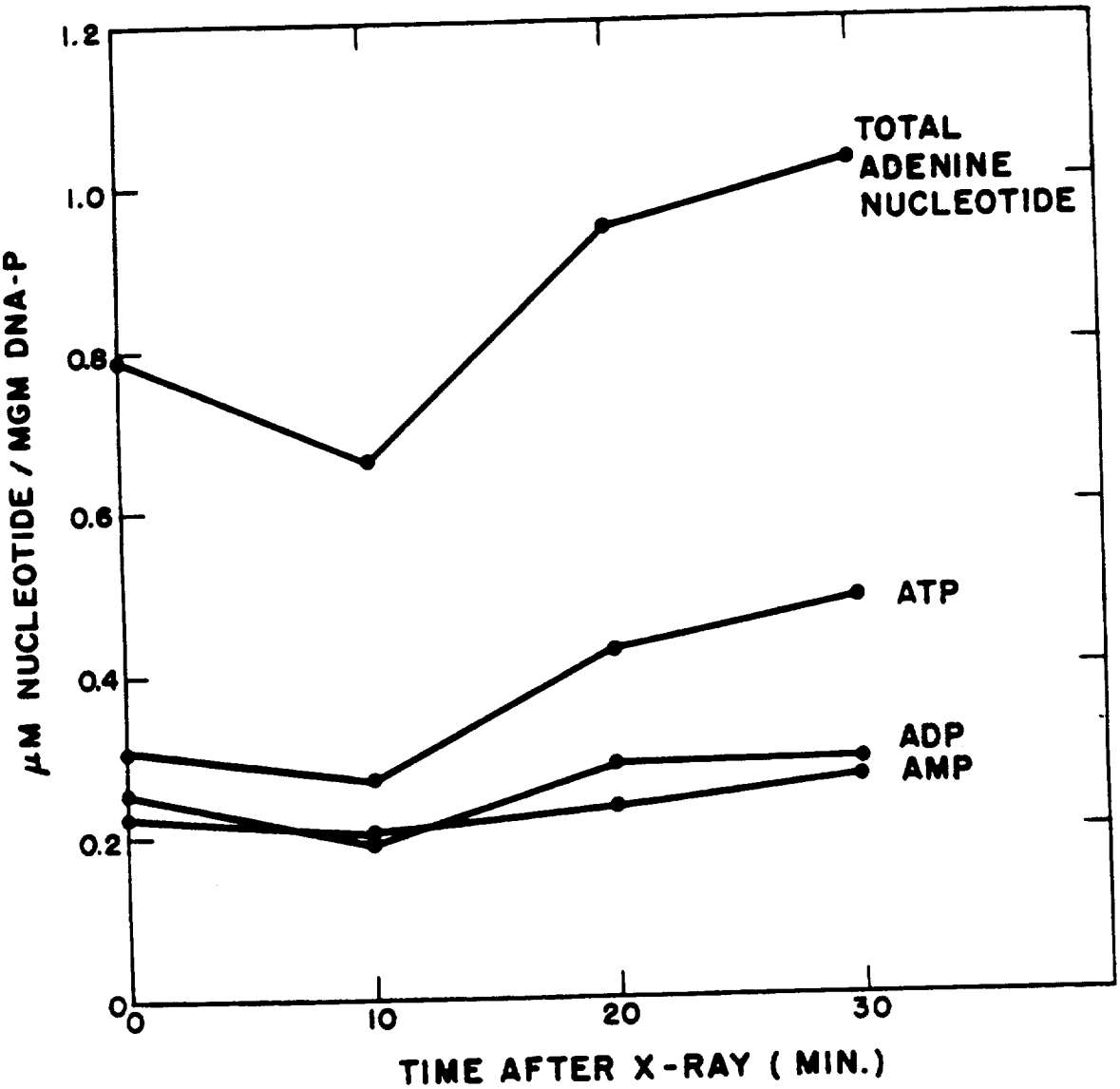


Fig. 1. Adenine nucleotide content of spleen nuclei following exposure to 200 rads.

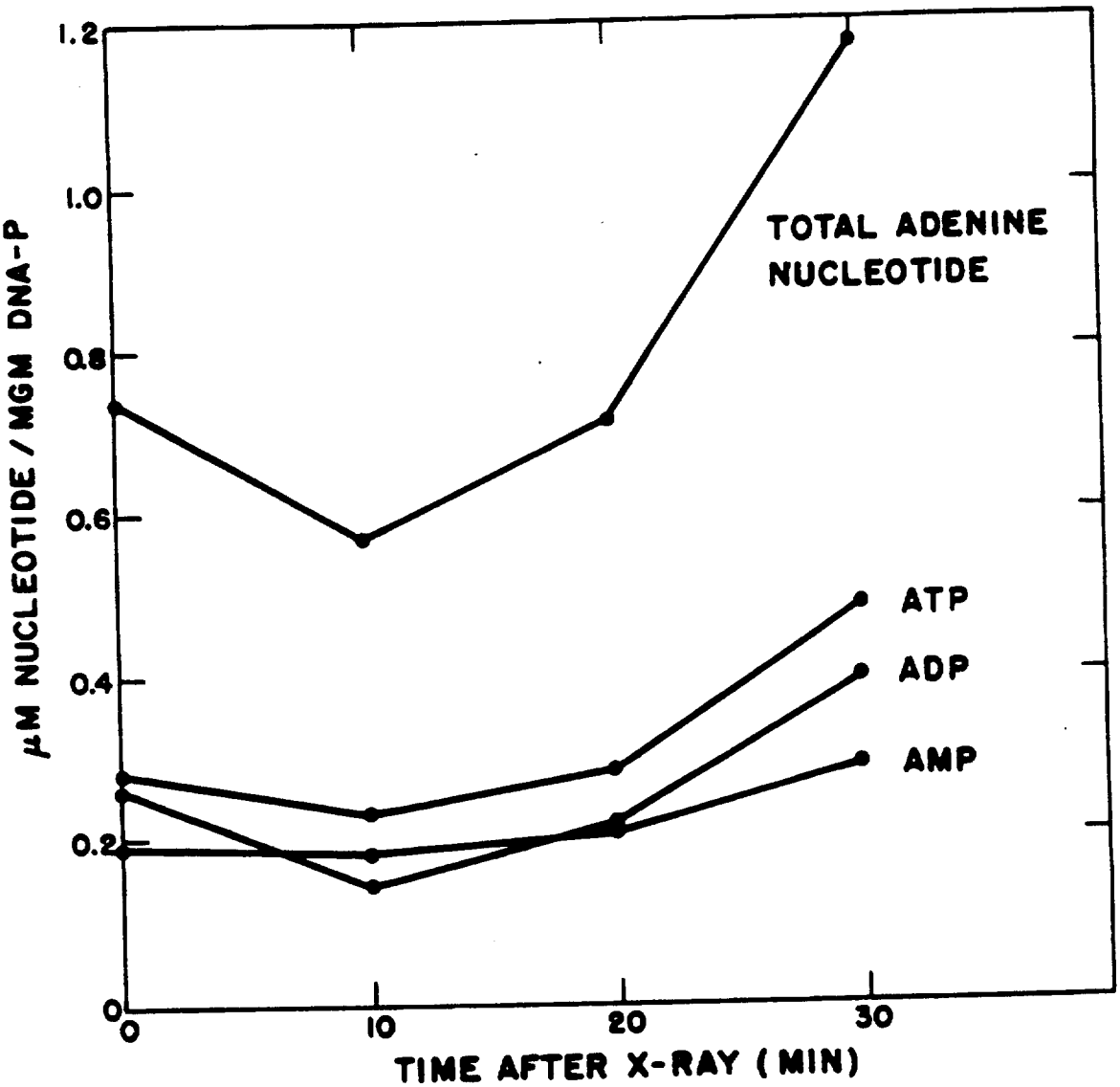


Fig. 2. Adenine nucleotide content of thymus nuclei following exposure to 200 rads.

Osawa, et al (3) have repeatedly emphasized that only bound nucleotides are phosphorylated and that neither added inorganic phosphate nor nucleoside monophosphates participate in phosphorylation of materials found in acid-soluble extracts following incubation at 0 or 38°C. However, van Bekkum (12) has demonstrated a decrease in the extent of labeling of total nucleotides of the spleen and thymus at 4 hours after irradiation. Thus, uptake of P^{32} by cytoplasmic phosphorylation processes might be expected to exchange with the nuclear system leading to labeling of nuclear nucleotides. Cytoplasmic nucleotides might, therefore, be considered a primary donor of phosphate for nuclear phosphorylation in vivo, but the process must proceed step-wise in order to explain phosphorylation in isolated nuclei where no cytoplasmic donor is present (2,3).

Table 2 summarizes results of a series of experiments in which 100 μc of P^{32}-orthophosphate was given immediately after irradiation (400 rads) and the animals sacrificed 20 minutes later. Spleen and thymus nuclei were rapidly isolated in sucrose-$CaCl_2$ containing cyanide, washed twice, extracted with trichloroacetic acid, and the nucleotides isolated chromatographically. It is apparent from these data that 20 minutes after exposure neither the adenine nucleotide nor deoxyribonucleic acid phosphorus content differed

TABLE 2. Phosphorus^{32} Labeling of Nuclear Adenine Nucleotides following in vivo Irradiation*

Tissue	Radiation Dose (rads)	µg DNA-P /Organ	µM Nucleotide /mg DNA-P		c/m/µM Nucleotide	
			ATP	ADP	ATP	ADP
Spleen	Control	73.3	1.70	1.05	3137	1590
Spleen	400	70.8	1.35	0.95	4978	1442
Thymus	Control	426.7	0.36	0.28	70,186	26,131
Thymus	400	359.7	0.37	0.34	61,486	28,479

*100 µc P^{32}-orthophosphate per animal.
Labeling time: 20 minutes.

appreciably from control values. The extent of labeling (expressed as c/m/μM of nucleotide) was also comparable, indicating that phosphorylation in the nuclei of irradiated intact cells failed to show the defect exhibited in the isolated nuclear system.

These attempts to demonstrate the nuclear phosphorylation defect in vivo have been uniformly unsuccessful. However, it must be emphasized that the system involves only nuclear and cytoplasmic ribonucleotides and that participation of deoxyribotides has yet to be demonstrated. Thus, implication of this radiation induced change in nuclear nucleotide metabolism must be subjected to further rigorous investigation regarding both the identity and origin of the nucleotides before it can be directly related to the defect in deoxyribonucleic acid synthesis of radio-sensitive cells.

REFERENCES

(1) W. A. Creasy and L. A. Stocken, Biochem. J. 69, 17 (1958).

(2) W. A. Creasy and L. A. Stocken, Biochem. J. 72, 519 (1959).

(3) S. Osawa, V. G. Allfrey, and A. E. Mirsky, J. Gen. Physiol. 40, 491 (1957).

(4) M. G. Ord and L. A. Stocken, Nature (London) 182, 1787 (1958).

(5) R. K. Crane and F. Lipmann, J. Biol. Chem. 201, 235 (1953).

(6) C. H. Fiske and Y. Subbarow, J. Biol. Chem. 66, 375 (1925).

(7) H. A. Krebs and R. Hems, Biochim. Biophys. Acta 12, 172 (1953).

(8) D. F. Petersen and A. Murray, III, Anal. Chem., in press (1960).

(9) E. Vischer and E. Chargaff, J. Biol. Chem. 176, 703 (1948).

(10) W. C. Schneider, J. Biol. Chem. 161, 293 (1945).

(11) H. M. Kier and J. N. Davidson, Arch. Biochem. Biophys. 77, 68 (1958).

(12) D. W. van Bekkum, Biochim. Biophys. Acta 25, 487 (1957).

Deoxynucleosides in the Urine of Irradiated Animals and Man

(D. F. Petersen, M. Magee, and A. Murray, III)

Introduction

Recent studies by Parizek, et al (1) demonstrated that increased amounts of deoxyribose-containing materials appeared in 24-hour urine specimens collected from irradiated rats. According to these authors, the major component of the Dische-positive material was deoxycytidine. In view of this report, it was of interest to investigate early changes in the deoxyribose content of the urine of irradiated animals and to identify the compounds contributing to the total deoxyribose observed. In addition to the animal experiments, it was possible to examine urine specimens from two chemical workers exposed to gamma-neutron irradiation in the most recent Los Alamos criticality accident.

Experimental

The urine specimens were chromatographed on Whatman No. 1 paper using two solvent mixtures: butanol-water (86:14 v/v) with an ammonia atmosphere, and isobutyric acid, 1M ammonia, and 0.1M EDTA (100;60:1.6 v/v/v). Spots were located in transmitted ultraviolet light (2) and the identity of each compound was established by (a) spectrophotometric analysis; (b) R_f value

compared with simultaneously developed authentic material; (c) homogeneity upon rechromatography with authentic material; (d) Dische reaction for deoxyribose; and (e) phosphorus analysis. Total deoxyribose was measured in urine by Dische's cysteine-sulfuric acid reaction (2) and identified on paper by Buchanan's modification (3). Quantitative estimations of purine and pyrimidine bases were performed spectrophotometrically as described by Vischer and Chargaff (4), and phosphorus was measured by the method of Fiske and Subbarow (5).

Results and Discussion

Total deoxyribose and deoxycytidine content of urine samples collected at frequent intervals after exposure to 200 and 400 rads are shown in Fig. 1. Increased excretion of both total Dische-positive material and deoxycytidine was detectable within 4 hours after exposure and increased steadily during the subsequent collection period. The deoxycytidine values are in quantitative agreement with the previous results of Parizek, et al (1) but inspection of Fig. 1 indicates that a large proportion of the deoxyribose-containing material cannot be accounted for as deoxycytidine. A similar situation was observed in the initial urine specimen obtained from the fatally irradiated patient 14 hours after he sustained an asymmetrical exposure to ~4000 to 12,000 rads of gamma-neutron irradiation. Total Dische-positive material was approximately

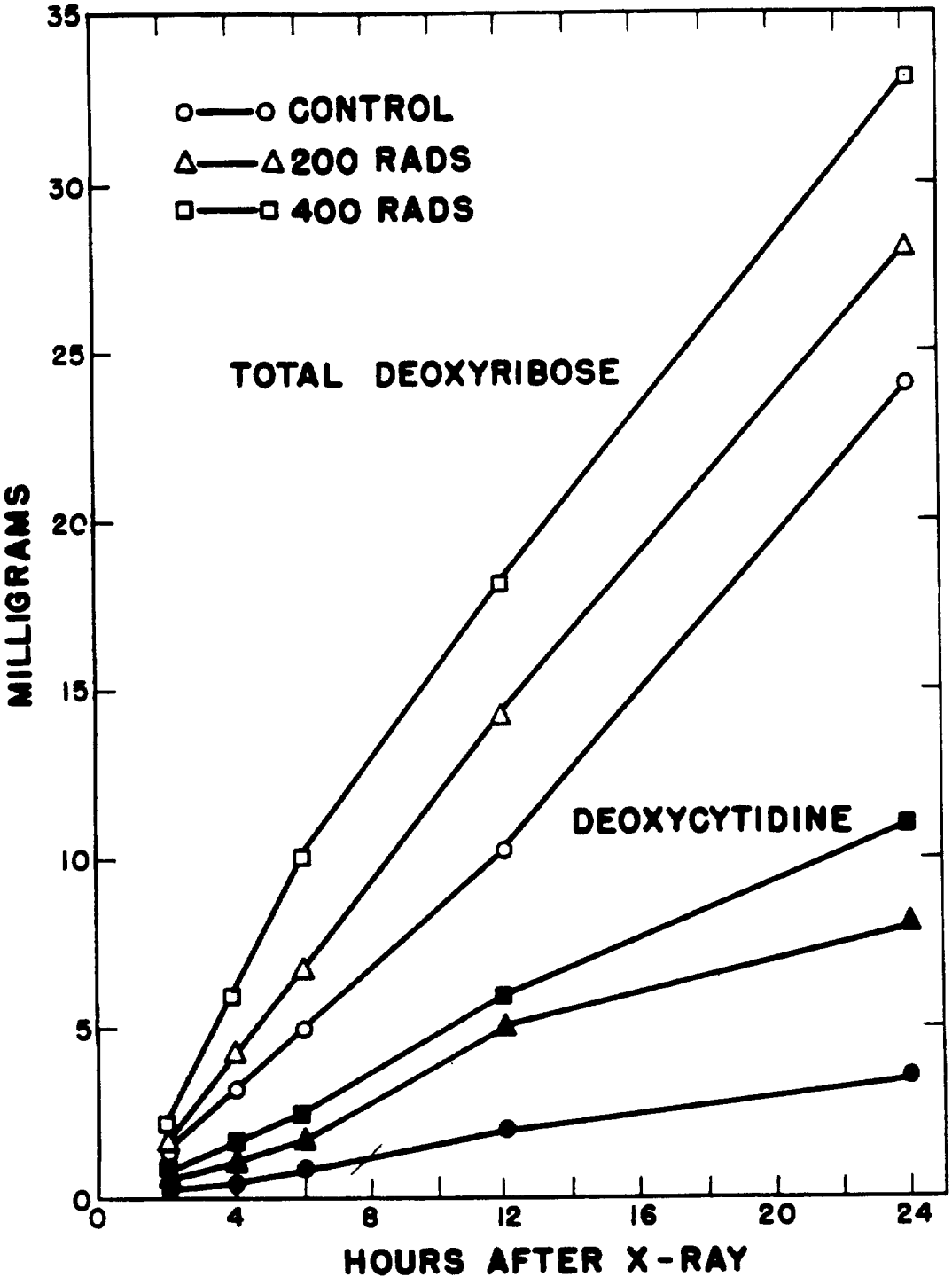


Fig. 1. Cumulative excretion of total deoxyribose and deoxycytidine in rat urine following whole-body X irradiation.

twice the amount found in specimens from a number of normal subjects.

A summary of the results of chromatographic analysis of the irradiated urine specimen is shown in Table 1. All the separated [redacted]ts were positive for deoxyribose; bands 1 and 2 were shown to contain phosphorus, and each identified compound migrated as a single homogeneous band when eluted and rechromatographed with authentic material. Band 5 is as yet unidentified, but preliminary data indicate that it is probably thymidine. That the appearance of deoxynucleosides of cytosine, adenine, and guanine is related to a defect in deoxyribonucleic acid (DNA) metabolism was further substantiated by identification of β-aminoisobutyric acid (BAIB), the major metabolite of thymidine (6), during characterization of the amino acids present in the irradiated specimen (7). In view of the appearance of significant amounts of BAIB, it is not surprising that the previous workers (1) reported failure to find increased amounts of thymidine. Appearance of deoxynucleotides has been attributed to gross radiation damage to the kidneys, and the possibility exists that the amount of phosphorylated nucleotide in urine may be a measure of such injury.

The relative amounts of nucleotide are presented as tentative data because the extent of recovery from the

TABLE 1. IDENTIFICATION OF DEOXYRIBOSE-CONTAINING COMPOUNDS IN HUMAN URINE FOLLOWING MASSIVE GAMMA-NEUTRON IRRADIATION

Band	Compound	R_f x 100 I	R_f x 100 II [a]	X_{max} pH 2	Relative Content (%) [b]
1	Thymidylic acid	--	38	265	18.5
2	Deoxyguanylic acid	--	46	255	16.4
3	Guanine deoxyriboside	19.2	56	257	9.3
4	Cytosine deoxyriboside	25.4	62	276	48.0
5	Unidentified	43.8	70	266	--
6	Adenine deoxyriboside	54.6	78	258	7.9

(a) Solvent I. Butanol-water (86:14 v/v), ammonia atmosphere.
Solvent II. Isobutyric acid, 1M ammonia, 0.1M EDTA (100;60;1.6 v/v/v).

(b) Calculated as per cent of the total µM of deoxyribose recovered in identified material.

preliminary charcoal adsorption step has been established only for deoxycytidine. Elution of tritiated deoxycytidine by pyridine-ethanol (v/v) was consistently 72 to 74 per cent after a single washing. Investigation of selective elution of each of the identified materials is currently in progress. No conclusion, therefore, has been drawn regarding the metabolic origin of the urinary deoxyribosides. However, it is anticipated that experiments employing labeled precursors will clarify this point.

REFERENCES

(1) J. Parizek, M. Arient, Z. Dienstbier, and J. Skoda, Nature (London) 182, 721 (1958).

(2) Z. Dische, Proc. Soc. Exptl. Biol. Med. 55, 217 (1944).

(3) J. G. Buchanan, Nature (London) 168, 1091 (1951).

(4) E. Vischer and E. Chargaff, J. Biol. Chem. 176, 703 (1948).

(5) C. H. Fiske and Y. Subbarow, J. Biol. Chem. 66, 375 (1925).

(6) K. Fink, R. B. Cline, R. B. Henderson, and R. M. Fink, J. Biol. Chem. 221, 425 (1956).

(7) D. F. Petersen and C. C. Lushbaugh, submitted to J. Occup. Med.

A Simple Intensification Screen for Ultraviolet Scanner Cameras (D. F. Petersen and A. Murray, III)

Detection of ultraviolet absorbing substances on paper chromatograms has been facilitated by preparation of a filter paper screen impregnated with 0.1 to 1 per cent ethanolic solutions of 1-methyl-4-(5-phenyl-2-oxazolyl)-pyridinium-p-toluene sulfonate (MY^4PO). The compound, developed in this laboratory by Ott, Hayes, and Kerr (1) as a liquid scintillator, was found to possess favorable excitation and emission characteristics for use in an apparatus of the type described by Drake, et al (2).

The device has been used primarily for detection of purine and pyrimidine nucleotide derivatives and has increased sensitivity from 25 to 50 gammas/cm^2 (quenching of incident ultraviolet from a mineralite lamp) to 0.5 to 1 gamma/cm^2. The paper intensification screens have been used for approximately one year without appreciable deterioration and thus satisfy requirements for stability, homogeneity, and extreme simplicity of preparation. Since the screens are quite thin, diffusion of fluorescence is eliminated and outlines are sharper than those obtained with a phosphor-coated glass screen.

Acknowledgment

The investigators are indebted to V. N. Kerr of the Biomedical Research Group for the sample of MY^4PO.

REFERENCES

(1) D. G. Ott, F. N. Hayes, and V. N. Kerr, J. Am. Chem. Soc. 78, 1941 (1956).

(2) N. A. Drake, W. J. Haines, R. E. Knauff, and E. D. Nielson, Anal. Chem. 28, 2036 (1956).

Biological Sulfur Activation Dosimetry (D. F. Petersen and W. H. Langham)

The high sulfur content of the keratinous appendages of the skin has been utilized to provide an estimate of the fast neutron dose in the most recent Los Alamos criticality accident. Due to the small amount of phosphorus relative to sulfur found in human hair (less than 1 per cent), P^{32} beta activity arises almost exclusively from the $S^{32}(n,p)P^{32}$ reaction. It was found that beta activity could be converted to incident dose of fast neutrons on the basis of 6 d/m/g hair per rad. Determinations performed on hair and sternal cartilage indicated respective incident doses of fast neutrons to the head and chest of ~2600 and 3000 rads (1). Current experiments suggest that due to the constancy of chemical composition and fixed anatomical location, a first approximation of the incident dose of neutrons above the sulfur threshold to various parts of the body may be determined simply by counting the beta activity of an ashed hair or nail sample. Sensitivity is of the order of 1 rad for samples weighing 10 g. A systematic investigation of the use of high sulfur content tissues and structures as a means of estimating fast neutron dose is being pursued.

Reference

(1) P. S. Harris, submitted to J. Occup. Med.

The Effect of MER-29 on Thiopental Sleep Time (D. F. Petersen, R. G. Gould, and E. H. Lilly)

Introduction

Several complex organic compounds have recently received considerable attention due to their capacity to suppress cholesterol biosynthesis and effect significant reductions in plasma cholesterol levels. Benzmalecene, the alpha isomer of N-(1-methyl-2,3-di-p-chlorophenylpropyl)-maleamic acid, and p-(di-N-propylsulfamyl)-benzoic acid (probenecid) introduced as potent renal tubular transport inhibitors affect cholesterol biosynthesis by inhibiting the first step, the condensation of acetate with CoA (1). Chronic administration of probenecid has been shown to cause transitory impairment of hepatic function (2), and benzmalecene has also been observed to cause liver damage in humans (3). MER-29 (1-[p(β-diethylaminoethoxy)phenyl]-1-(p-tolyl)-2-(p-chlorophenyl)ethanol) markedly decreases cholesterol biosynthesis by inhibiting the last step, the reduction of the side chain double bond of 24-dehydrocholesterol (4). In view of the hepatic involvement noted previously (2), it was of interest to determine if MER-29 has any effect on liver function tests. Hepatic function markedly influences the duration of anesthesia resulting from administration of barbiturate analogs primarily detoxified by the liver. Thiopental

was chosen for this experiment because, in addition to its detoxification by the liver and kidneys, it is rapidly sequestered by body fat (5), and a significant aberration in lipid metabolism induced by chronic MER-29 feeding might thus be detected.

Experimental

Female RF mice weighing 20 g at the beginning of the experiment were divided into four groups, each containing 20 animals. Each group was given tap water and fed ad libitum a diet as outlined in Table 1. On the tenth and fortieth days of feeding each animal was given 30 mg/kg thiopental intravenously, and the duration of anesthesia was recorded. Return of the righting reflex in a quiet room was taken as the end point of the test.

Results and Discussion

The mean duration of anesthesia for each group is shown in Table 2. These data demonstrate that while a single subcutaneous injection of carbon tetrachloride caused a marked and persistent increase in sleeping time, levels of MER-29 which effected a pronounced decrease in plasma cholesterol (6) and weight gain due to anorexia failed to alter significantly duration of thiopental anesthesia. It was, therefore, concluded that short-term chronic feeding of MER-29 does not cause alterations in hepatic function measured by this test.

TABLE 1. Experimental Diets

Group	No. of Animals	Composition of Diet
I	20	Powdered Purina chow + 5% corn oil
II	20	Powdered Purina chow + 5% corn oil (2 g/kg CCl_4 injected subcutaneously)
III	20	Powdered Purina chow + 5% corn oil 0.05% MER-29
IV	20	Powdered Purina chow + 5% corn oil 0.1% MER-29

TABLE 2. Thiopental Sleep Time following Chronic MER-29 Feeding

Group	Duration of Anesthesia (minutes)		
	Wt.	Mean	% of Control
10 days - Time Tested			
I Control	21.5	3.93	100
II CCl4 Control	19.6	15.02	383
III 0.05% MER-29	22.0	3.97	101
IV 0.1% MER-29	20.3	4.35	111
40 days - Time Tested			
I Control	25.8	4.85	100
II CCl_4 Control	24.0	9.38	193
III 0.05% MER-29	25.3	5.38	111
IV 0.1% MER-29	23.6	5.47	113

REFERENCES

(1) V. D. Wiebelhaus, K. H. Beyer, H. F. Russo, F. T. Brennan, and E. R. Wynosky, Fed. Proc. 12, 155 (1953).

(2) S. H. Blondheim, J. Appl. Physiol. 7, 529 (1955).

(3) I. H. Page, personal communication (1959).

(4) J. Avigan, D. Steinberg, M. J. Thompson, and E. Mosettig, Biochem. Biophys. Res. Comm., in press (1960).

(5) B. B. Brodie, F. Bernstein, and L. C. Mark, J. Pharm. Exptl. Therap. 105, 421 (1952).

(6) R. G. Gould, E. H. Lilly, and V. E. Mitchell, Circulation, in press (1960).

Effects of MER-29 (1-[p-(β-Diethylaminoethoxy)-phenyl]-1-(p-tolyl)-2-(p-chlorophenyl)ethanol) on Cholesterol Concentration in Plasma and Tissues and on Cholesterol Biosynthesis in vivo and in vitro in Rats (R. G. Gould, V. Mitchell, and E. H. Lilly)

An intensive study has been made of the effect of feeding a new drug MER-29 to rats on the cholesterol concentrations in plasma, liver, intestine, adrenals, kidneys, and residual carcass. When fed at a level of 0.05 per cent of the diet, this drug decreases cholesterol (as determined colorimetrically by the Lieberman-Bruchard reaction) by about 50 per cent without producing any evidences of toxicity or undesirable side effects except for a decrease in rate of growth due to decreased food intake. Analyses for total sterols by a gravimetric method have not revealed the accumulation of larger than normal amounts of sterols. However, the isolated sterol is not as nearly pure cholesterol as in control animals, as shown by melting point, etc.

It has also been found that MER-29 greatly accelerates the disappearance of stored excess cholesterol in the livers of rats, previously fed cholesterol for 28 days, and its effect on atheromatous lesions in rabbit arteries is under study.

MER-29 also has some effect in inhibiting cholesterol absorption. The inhibitory effect of MER-29 on cholesterol

biosynthesis has been demonstrated using mevalonic acid-2-C^{14} as a precursor in intact rats and in homogenates from MER-29 fed rats. In addition, marked inhibition has been shown to result from addition of very small amounts of the drug to homogenates from normal rat livers.

A method of analysis for MER-29 in tissues has been developed which is simple, specific, and sensitive. Less than 10 mg can be measured in a large sample of plasma or tissue. The method is based on the fact that MER-29 can be first extracted into the nonsaponifiable fraction and then extracted into a small volume of dilute acetic acid. Although MER-29 itself does not absorb at 314 mμ, it is readily converted to an anhydro-derivative by acidification with HCl, which absorbs very strongly at this wavelength. The change in optical density following addition of HCl is a measure of the MER-29 present in the tissue.

Effects of MER-29 (1-[p-(β-Diethylaminoethoxy)-phenyl]-1-(p-tolyl)-2-(p-chlorophenyl)ethanol) on Plasma Cholesterol Levels and Steroid Hormone Metabolism in Humans (R. G. Gould, B. Bloom, W. Hentel, R. G. Schoenfeld, and W. Taylor of the Veterans Hospital in Albuquerque)

A study of the effect of 250 mg per day of MER-29 on plasma cholesterol levels of a group of 17 patients at the Veterans Hospital in Albuquerque, New Mexico, has been in progress for several months. The primary aim is to investigat the effect of the drug on atherosclerotic plaques. It is anticipated that a number of these patients, selected for a limited life expectancy, will die during the course of the study and that it will be possible to obtain data on cholesterol concentrations in plaques and in all body tissues. Up to the present, decreases of about 25 per cent on the average in the plasma cholesterol levels have been noted. No significant changes in 17-ketosteroids or in 17-hydroxycorticosteroids have been observed, indicating that this drug does not seriously interfere with adrenal-cortical hormone biosynthesis at this dosage level.

Effect of Adrenalectomy, Hypophysectomy, and Cholesterol Feeding on the Radiation-Induced Increase in Hepatic Cholesterol Biosynthesis (R. G. Gould, E. H. Lilly, and V. L. Bell)

It has been shown that the rate of hepatic biosynthesis of cholesterol in rats as measured by the incorporation of acetate-1-C^{14} in vivo is decreased by both adrenalectomy and hypophysectomy and that whole body X irradiation increased the rate in adrenalectomized rats but not in hypophysectomized rats, suggesting that the pituitary is concerned in mediating the effect of radiation on cholesterol biosynthesis.

Rats fed diets high in cholesterol accumulated large amounts in liver, particularly in the esterified fraction. Whole body radiation mobilized this stored cholesterol very rapidly and caused its disappearance. It also increased the rate of synthesis proportionately as much as in normal animals, although the actual rates were very much less.

All the data obtained in this investigation on rate of biosynthesis as a function of the concentration of cholesterol in liver support the previously reported hypothesis that the log of the synthetic rate is inversely proportional to the concentration of free (i.e., unesterified) cholesterol with a slope corresponding to a doubling of the synthetic rate or a decrease of 0.12 to 0.15 mg of cholesterol per gram of liver.

The Metabolism of Diethylenetriamine Pentaacetic Acid (DTPA)

(H. Foreman and M. Magee)

Introduction

Because diethylenetriamine pentaacetic acid (DTPA) appears to be an agent of promise in the treatment of heavy metal poisoning and in other biological applications, it was decided to investigate its behavior in the body. The compound was labeled with C^{14} by A. Murray of this Laboratory. Metabolic studies using the labeled material were carried out after oral, intramuscular, intravenous, and intraperitoneal administration to rats.

Results

The behavior of this compound in the body was similar to that of Ca EDTA, with some minor quantitative differences. The compound was rapidly excreted, primarily in the urine. Over 90 per cent of parenterally administered doses appeared in the urine by 24 hours. Most of the dose was cleared from the blood and tissue fluids with a half-time of 35 minutes. However, a portion of the dose was slowly cleared with a half-time of 1 day (Fig. 1).

Tissue distribution of the labeled drug at 24 hours is presented in Table 1. A larger proportion of parenterally

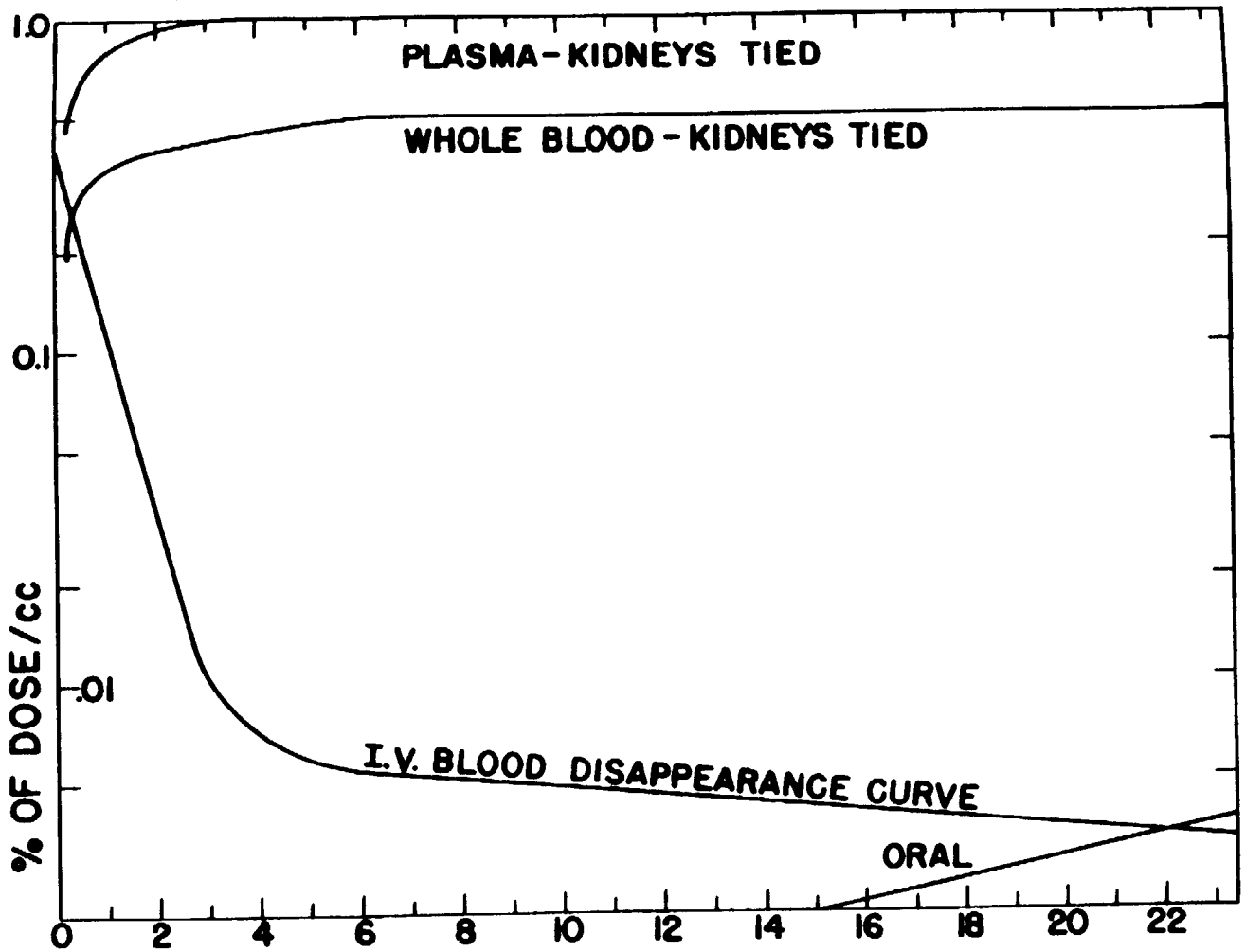


Fig. 1. Clearance from blood and tissue fluids after injection of Ca DTPA.

TABLE 1. Distribution of C^{14}-labeled Ca DTPA in Rats at 24 Hours after Administration[a]

Tissue or Sample	Intra-venous	Intra-muscular	Intra-peritoneal	Oral[b]	Specific Activity[c]
Urine	87.3	91.9	91.0	3.60	10
Feces	5.4	3.8	3.3	75.00	--
Kidney	0.7	0.3	0.4	0.10	174
Liver	0.3	0.8	0.2	0.05	--
Gastro-intestinal Tract	--	--	--	18.30	110
Remains	5.7	2.6	5.2	0.51	12

[a]Results are expressed in per cent of recovered dose.

[b]Sacrificed at 48 hours.

[c]Specific activity is the c/m/gm of tissue.

administered doses was found in the body at 24 hours than was the case with Ca EDTA (i.e., over 5 per cent as compared to less than 2 per cent). This may account for the more prolonged effect of DTPA when used to hasten excretion of metal ions from the body. At 24 hours, the residual compound was largely concentrated in the kidneys and liver.

The dilution volume shortly after injection was calculated from data illustrated in Fig. 1. When the urinary excretion was blocked by tying off the kidneys, whole blood and plasma concentrations leveled off and remained constant for the duration of the study (i.e., 24 hours). The plasma concentration reached 1 per cent of the injected dose per cc, indicating a dilution volume of 100 cc (i.e., approximately 30 per cent of the body weight of the rats used). It is likely that the fast component in the blood disappearance curve represented equilibrium in the extracellular fluids and the slow component is related to equilibrium of material between blood and tissue substance.

It is of interest that similarly to EDTA, the material does not pass into the red cells. Renal clearance, calculated from the blood clearance data, is 2 cc/min and indicates that excretion occurs both by glomerular filtration and tubular secretion.

Studies in which the drug was administered by stomach

tube indicated that the material was poorly absorbed. In 48 hours, 75 per cent of the dose appeared in the feces and 18 per cent was still in the gastrointestinal contents. As judged by the appearance of activity in the urine and tissues, less than 4 per cent was actually absorbed. Very low blood levels after oral administration verified this finding (Fig. 1).

Little of the activity ever appeared in the respiratory CO_2, which suggested that practically none of the compound was oxidized. Chromatography provided further evidence for stability of the material in the body. The presence of a single band on autoradiographs of plasma, urine, and fecal extracts indicated that the radioactivity was associated with a single compound. That this compound was DTPA was demonstrated by showing this material to have the same R_f value as an authentic sample of the original DTPA.

The Effect of Diethylenetriamine Pentaacetic Acid (DTPA) on Acceleration of Plutonium Excretion (H. Foreman, M. B. Roberts, and M. Magee)

Introduction

Screening experiments done in this laboratory and in others (1-4) have shown that DTPA is considerably superior to EDTA in hastening excretion of plutonium, both when the drug is given a short time after the plutonium administration and when it is given long after the plutonium (Table 1). In view of these interesting observations, follow-up studies were done to investigate the in vivo chelating potential of DTPA more intensively.

Methods and Results

The present studies were carried out using Am^{241} as a model for plutonium. $Americium^{241}$ was used because it behaves very similarly to plutonium in the body and because it is a gamma emitter which can be used to measure residual activity in the intact animal by use of a small animal counter (5). The isotope was injected intravenously, and at various time periods later DTPA was administered parenterally in some animals (200 mg/kg) and by oral administration (150 mg/kg) in others. Retention of the isotope was followed by serial counting, daily at first, and less frequently later

TABLE 1. Effects of Acute and Chronic DTPA Treatment on Excretion and Deposition of Pu^{239}

	Urine	Feces	Liver	Spleen	Remains	Skeleton
Acute DTPA Study[a]						
Controls	12.70	7.06	5.86	0.80	20.11	53.36
DTPA	86.20	4.05	0.40	0.10	3.70	5.83
Chronic DTPA Study[b]						
Controls	7.7	28.9	3.5	---	9.99	49.5
1 Week	20.6	21.2	4.2	1.8	7.30	44.8
2 Weeks	25.4	29.8	1.1	---	6.50	37.0
4 Weeks	39.9	34.6	0.4	---	2.80	22.3
6 Weeks	37.0	27.3	0.3	---	3.40	30.7

[a] In the acute study, the drug was started 2 hours after the isotope was given and continued daily for 4 days.

[b] In the chronic study, the drug was started 1 month after the isotope was given and continued for periods indicated in the table.

in the small animal counter. Figure 1 shows the times of administration of the drug, as well as results of the treatment.

Several features are apparent in the effect of administration of the drug on retention of the isotope.

1. When injected daily, starting 2 hours after the isotope was given, the drug is quite effective at first so that after 2 weeks only 7 per cent of the administered dose was still in the body. This verifies the initial observations with plutonium. However, continued administration of the drug brought forth less and less of an increase in americium excretion.

2. After oral administration, in which the drug was mixed with drinking water, accelerated excretion was also noted. However, this was not as great as occurred when the drug was given by injection. After 2 weeks, 12 per cent of the initial dose was still in the body. Here too the drug lost its effectiveness with repeated administration.

3. When parenteral administration was started at various time periods after the isotope was given, the effectiveness of treatment was directly dependent on the delay in treatment; the longer the delay, the less the effectiveness. When the drug was started 1 week after the isotope was given, the residual isotope was 12 per cent. Compared to the situation

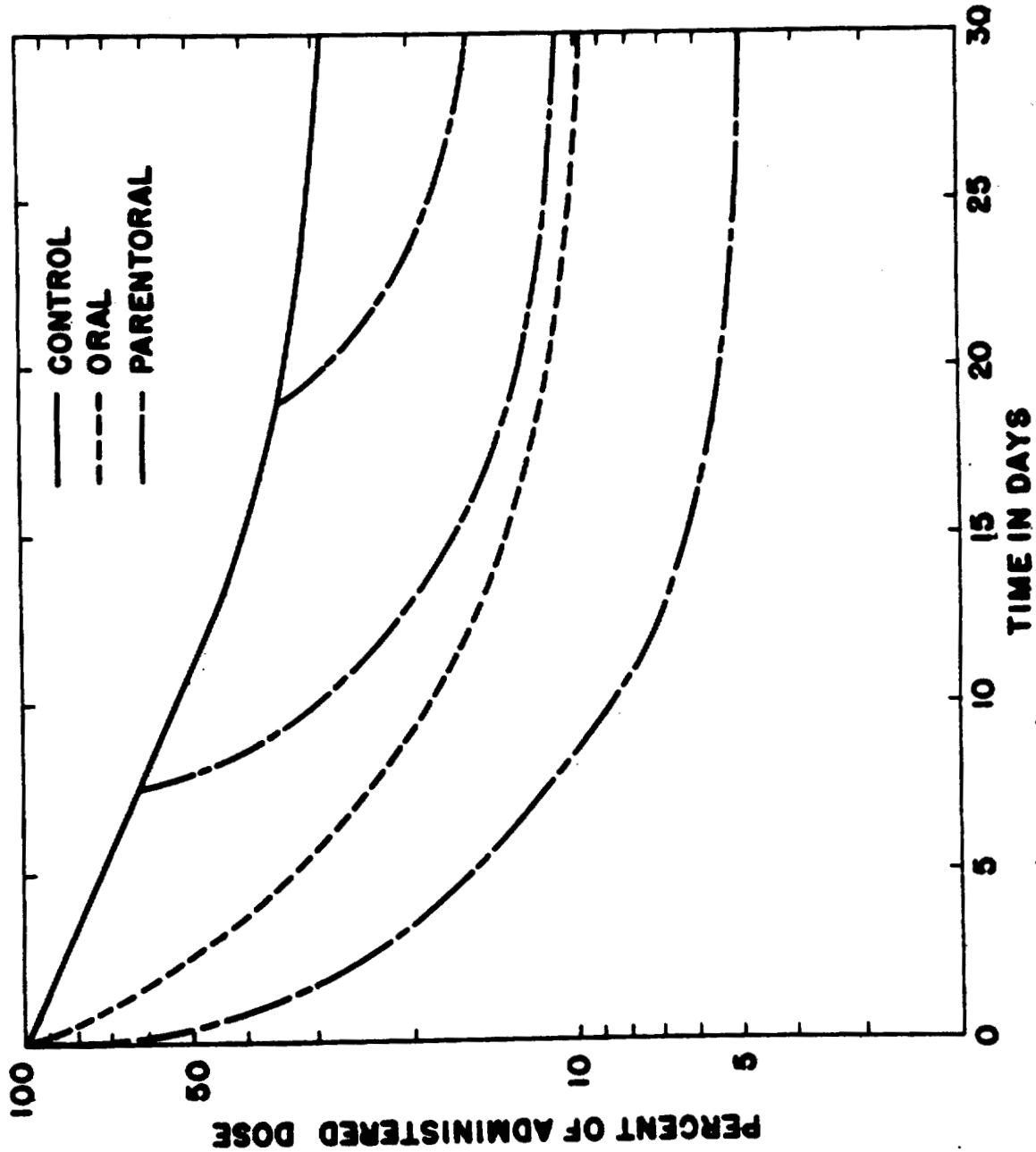


Fig. 1. Americium retention at various time periods after oral and parenteral administration of DTPA.

when the treatment was started at 16 days, the residual isotope was 15 per cent. Both of these can be compared to a residuum of 30 per cent of the initial dose, at this same time period, in the controls.

It appears that the decrease in effectiveness of the drug with repeated administration is related to at least two factors, namely the natural decrease in availability of the isotope (i.e., the increase in the amount of isotope that is fixed to tissue with time), and because the rate of excretion of the chelated metal is far greater than the rate of mobilization of the metal from the tissues (even under the effect of therapeutic agent), and hence the pool of available metal becomes exhausted and time is required for its replenishment. The practical significance of these observations is that in all probability less frequent administration of the drug at late time periods is just as effective as daily administration.

References

(1) A. Catsch and Le Du Khuong, Nature 180, 609 (1957).

(2) H. Kroll, S. Korman, E. Siegel, H. E. Hart, B. Rosoff, H. Spencer, and D. Laszlo, Nature 180, 920 (1957).

(3) V. H. Smith, Nature 181, 1792 (1958).

(4) J. F. Fried, E. H. Graul, J. Schubert, and W. M. Westfall, Atompraxis 5, 1 (1959).

(5) K. T. Woodward, C. R. Richmond, and W. Langham, Proceedings of the Health Physics Society, 79 (1956).

Batyl Alcohol and Radiation Damage (H. Foreman, M. B. Roberts, and M. Magee)

Introduction

Over the past few years evidence has accumulated which indicates the existence of humoral stimulatory factors which exert important physiological and patho-physiological regulatory control of erythropoiesis. Two factors appear to be implicated in the homeostasis of red blood cell levels. One apparently controls the rate of erythroblastic cellular division and the other the synthesis of hemoglobin. Linman, Bethell, and Long (1) have presented evidence which indicates that α-octadecylglycerolether (batyl alcohol) is a naturally occurring material which influences erythroblastic cellular division. It appeared to us that because of its bone marrow stimulatory effect, batyl alcohol might be effective in ameliorating radiation damage. Indeed, a search through the literature showed that Edlund (2) demonstrated a protective effect of this compound in mice after whole body irradiation. The positive results presented in that paper suggested that perhaps batyl alcohol could be used to potentiate the therapeutic effects of transfused bone marrow given after irradiation.

Methods and Results

Preliminary experiments in the form of 30-day lethality studies were carried out. Female RF mice (approximately 30 to a group) aged 80 days were given whole body X irradiation at several dose levels. Five groups of animals were irradiated at each dose level. One group at each dose was kept as control, and each of the other groups was given a different post irradiation treatment. The different treatment schemes are indicated in Table 1.

The results at the various dose levels varied considably, from showing just a little protective effect to markedly effective results. Table 1 shows the results in the most successful experiment, and are sufficiently encouraging to warrant further investigation. The variation in results is somewhat disturbing, but apparently lack of reproducibility is a weakness in many radiation protective experiments reported in the literature.

References

(1) J. W. Linman, F. H. Bethell, and M. J. Long, J. Lab. Clin. Med. 52, 596 (1958).

(2) T. Edlund, Nature 174, 1102 (1954).

TABLE 1. Batyl Alcohol as Protection against Irradiation

Group	Treatment	Animals (No.)	Survival	Survival (%)
I	525 r X irradiation	27	0	0
II	525 r + corn oil	25	2	8
III	525 r bone marrow + corn oil	27	3	11
IV	525 r + batyl alcohol in corn oil (25 mg/day)	28	8	29
V	525 r + bone marrow + batyl alcohol in corn oil (25 mg/day)	27	14	52

Nephrotoxicity of Diethylenetriamine Pentaacetic Acid (DTPA)

(H. Foreman, C. C. Lushbaugh, M. Magee, and G. Humason)

Introduction

In view of the kidney damage seen after administration of ethylenediamine tetraacetic acid (EDTA), it was decided to carry out studies to determine the nephrotoxic potential of diethylenetriamine pentaacetic acid (DTPA), a compound with related structure and action. Both compounds are of considerable current interest as chelation agents for enhancement of elimination of radioisotopes from the body.

Methods and Results

The study was carried out in a manner similar to an earlier one with Ca EDTA (1). Male Sprague-Dawley rats were injected intraperitoneally at various dose levels to 30 to 100 mg/kg, daily for 16 days, with a neutral solution of Ca DTPA containing 1 per cent procaine. At that time the animals were sacrificed and pieces of kidney were fixed in 10 per cent formalin. Tissues were also taken for frozen section. Fixed tissues were eventually stained with hematoxylin and eosin and by the periodic acid-Schiff's procedure. The frozen sections were stained with oil red O for fat.

A comparison of the kidney changes caused by DTPA with those of EDTA is difficult because of the apparently different

lesions caused by the two drugs. EDTA, as previously described (1), produces a reversible osmotic nephrosis which, when given in large concentrations and over long periods of time, can progress fatally. In EDTA poisoning the proximal nephron is affected by intracellular distention caused by water. The water simulates vacuoles in appearance, since it accumulates in varying sized droplets between the cytoplasmic granules and is unstainable. When this difference is severe, the cellular walls rupture and the tubules are found filled with amorphous debris and lined by cells which have only occasional nuclei and occasional wisps of remaining cell wall. DTPA, on the other hand, produces a "hyaline granular" type of lesion in all of the animals studied, even at very low doses. Accompanying this hyaline droplet change (Fig. 1), there is a cytoplasmic shedding which leads to formation of light hematoxylinaphilic, amorphous casts. True cytoplasmic vacuolization and rupture, as seen in the EDTA kidneys, does not occur until very high doses are given (500 mg DTPA/kg). When these vacuolar changes are seen with DTPA, the hyaline droplet change is apparently suppressed.

The hyaline droplet is destructive in its own right. As the aqueous vacuoles in EDTA rupture cells, so do the large hyaline droplets caused by the relatively low doses of DTPA (i.e., the 120 to 250 mg/kg range, see Fig. 1). The clinical

Fig. 1. Histopathological effects of DTPA.

importance of these two types of osmotic nephrosis is debatable. There is no doubt that the vacuolar type can seriously interfere with renal function. While clinical manifestations associated with the hyaline granular type have not as yet been recorded, this lesion would appear potentially to be serious in view of the widespread tubular degeneration, obstruction, and necrosis.

Reference

(1) H. Foreman, C. Finnegan, and C. C. Lushbaugh, J. A. M. A. 160, 1042 (1956).

Retention and Excretion of Radionuclides of the Alkali Metals by Five Mammalian Species (C. R. Richmond)

Introduction

Much of the data incorporated into calculations of maximum permissible concentrations of radionuclides in man and in air and water are obtained from small mammals. Usually the data are substituted directly and seldom are they extrapolated on the basis of interspecific metabolic or physiologic correlations. This study is part of a general program designed to investigate the comparative retention, excretion, and absorption of radionuclides in several species of mammals by whole body in vivo radioassay techniques. Data are obtained from man whenever practicable. Although these results were reported earlier (1), the present report includes some extension of the data and additional interpretation with regard to interspecies correlations.

Methods and Results

Large volume liquid scintillation detectors were used to measure whole body retention and excretion of Na^{22}, Rb^{86}, and Cs^{134} or Cs^{137} in 5 species of mammals after acute oral or parenteral administration of 0.1 to 1.0 μc doses. Single or multiple component exponential functions of the form

$$R_t = \sum_{i=0}^{n} \left[a_i e^{-k_i t} \right]$$

best fit the whole body retention data.

Figures 1, 2, and 3 show families of retention functions of radiosodium, radiorubidium, and radiocesium for the species studied. The area under each curve, which is proportional to the number of disintegrating atoms and therefore related to dose, increases from mouse to man for all 3 radionuclides.

Parameters of the retention functions of the 3 radionuclides for the 5 species are given in Table 1. These values were determined by the somewhat subjective method of graphic analysis. The original data are now being analyzed by a machine method which performs iterative least squares fittings to a sum of exponentials.

In man, the mean biological half-time of the longest component was ~100 days for Rb^{86}, and ~140 days for Cs^{134} or Cs^{137}. These components represented ~99 per cent of the total area under the composite retention functions. Most of the Na^{22} administered to man is retained with a biological half-time of ~11 days. Consequently, most of the total area of the retention function (90 per cent) is contributed by the first 2 components. The longest component (~445 days in man) represents only about 0.3 per cent of the initial dose, yet

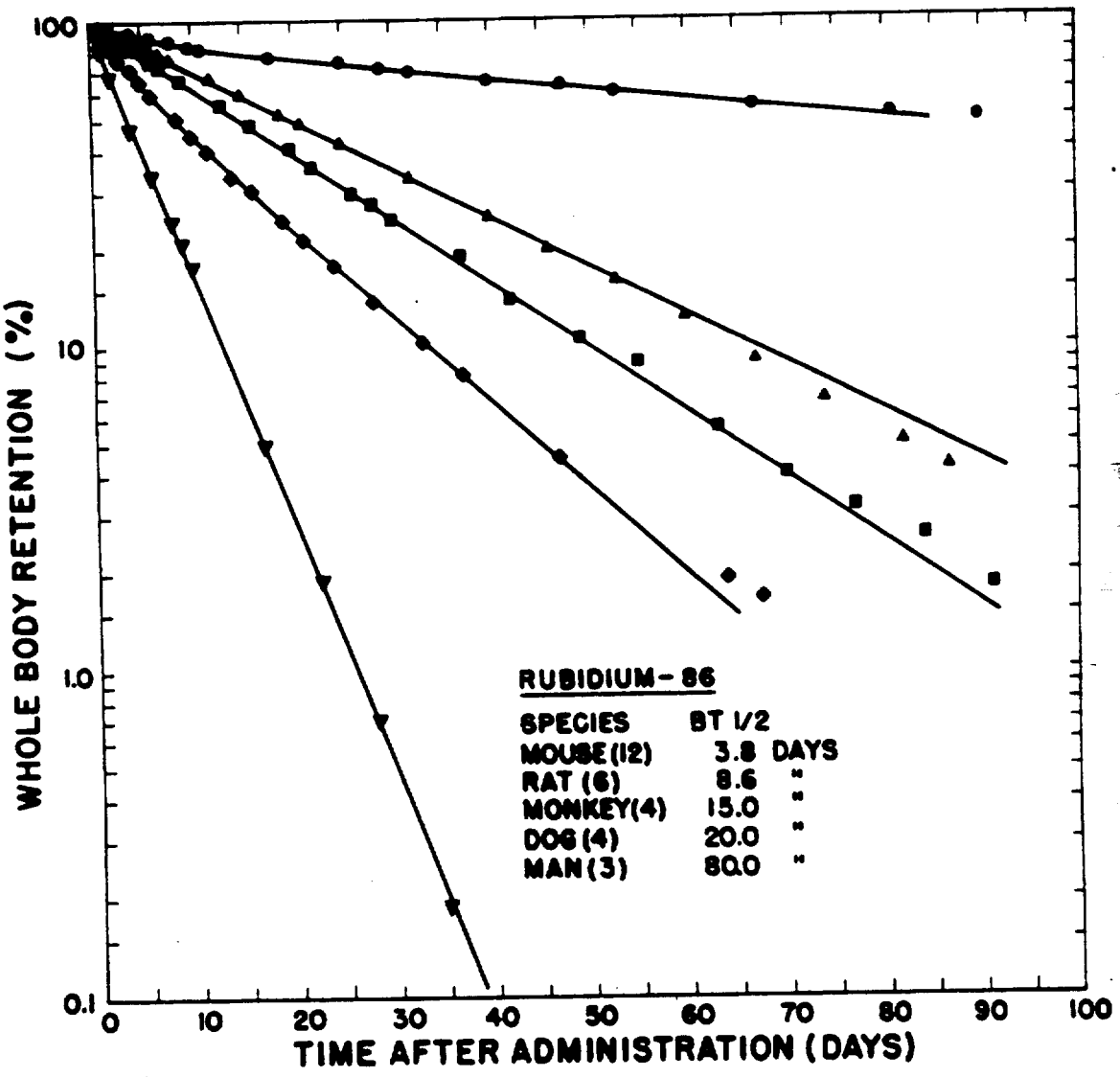


Fig. 2. Pattern of Rb^{86} retention in five species of animals.

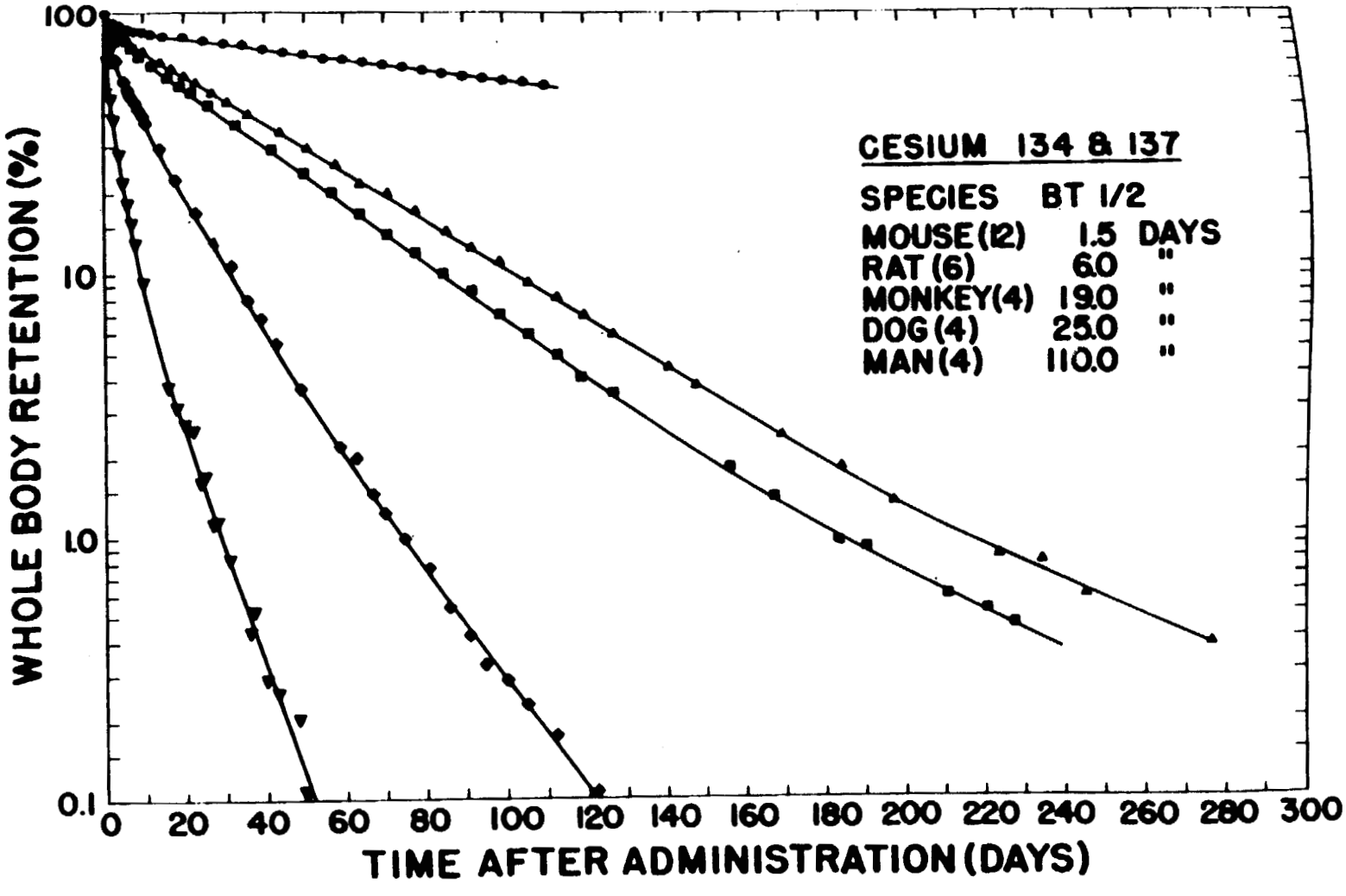


Fig. 3. Pattern of Cs^{134} and Cs^{137} retention in five species of animals.

TABLE 1. Parameters of the Retention Functions* of Na^{22}, Rb^{86}, and Cs^{137} in Mice, Rats, Monkeys, Dogs, and Man

Nuclide	Species	Route	Component 1 a	Component 1 $T_{1/2}$	Component 2 a	Component 2 $T_{1/2}$	Component 3 a	Component 3 $T_{1/2}$
Na^{22}	Mouse (12)	Oral	84.6	1.5	15.0	2.5	0.4	45.0
	Rat (6)	Oral	84.0	2.5	15.0	6.0	1.0	105.0
	Monkey (4)	I. V.	58.8	5.5	40.0	9.0	1.2	120.0
	Dog (4)	I. V.	56.3	7.5	43.0	10.0	0.7	205.0
	Man (3)	Oral	48.7	8.5	51.0	13.5	0.3	445.0
Rb^{86}	Mouse (12)	Oral	100.0	3.8	---	---	---	---
	Rat (6)	Oral	20.0	3.5	80.0	11.5	---	---
	Monkey (4)	I. V.	100.0	15.0	---	---	---	---
	Dog (4)	I. V.	100.0	20.0	---	---	---	---
	Man (3)	Oral	12.0	4.5	88.0	98.5	---	---
Cs^{137}	Mouse (12)	Oral	48.5	0.5	36.0	2.4	15.5	6.5
	Rat (6)	Oral	17.0	0.8	41.0	6.8	42.0	13.5
	Monkey (4)	I. V.	18.0	3.0	60.0	23.0	22.0	40.5
	Dog (4)	I. V.	14.0	1.1	56.0	27.0	30.0	43.5
	Man (4)	Oral	10.0	5.0	90.0	140.0	---	---

*$R_t = \sum_{i=o}^{n} \left[a_i e^{-0.693t/(T_{1/2})_i} \right]$, where t and $T_{1/2}$ are expressed in days.

it accounts for ~10 per cent of the total area. This finding emphasizes the need for retention data over long periods and points out the need for extremely sensitive detection systems. The components with relatively long half-times observed for all animals given Na^{22} presumably represent slow exchange of bone sodium.

The whole body is the critical organ for all 3 radionuclides. Gastrointestinal absorption for all 3 radionuclides is essentially 100 per cent. Values for $(MPC)_w$, calculated from human retention data, were 8.2×10^{-3} μc/ml for Na^{22}, 1.9×10^{-3} μc/ml for Rb^{86}, and 2.2×10^{-4} μc/ml for Cs^{137}.

One of the primary objectives of these studies is to look for interspecies correlations to provide a more sound basis for extrapolation of metabolic data from the more common laboratory animals to man. In an earlier report, the biological half-time $BT_{1/2}$ (i.e., the time required to excrete the first half of an administered dose) was shown to correlate with the body surface area (SA) according to the expression

$$\log BT_{1/2} = \log k + b \log SA$$

In the present study, the effective area of the retention function (i.e., the total area under the retention curve) for the 3 radionuclides in the 5 species was correlated with the

body weight (Fig. 4). Although the log-log plot is relatively insensitive to fluctuations of the variable parameters, the data definitely show that extrapolation from lower animals to man on the basis of the relationship to body weight would be more accurate than direct substitution of the values of any of the 4 other species.

Reference

(1) C. R. Richmond, Retention and Excretion of Radionuclides of the Alkali Metals by Five Mammalian Species, Los Alamos Scientific Laboratory Report LA-2207 (1958).

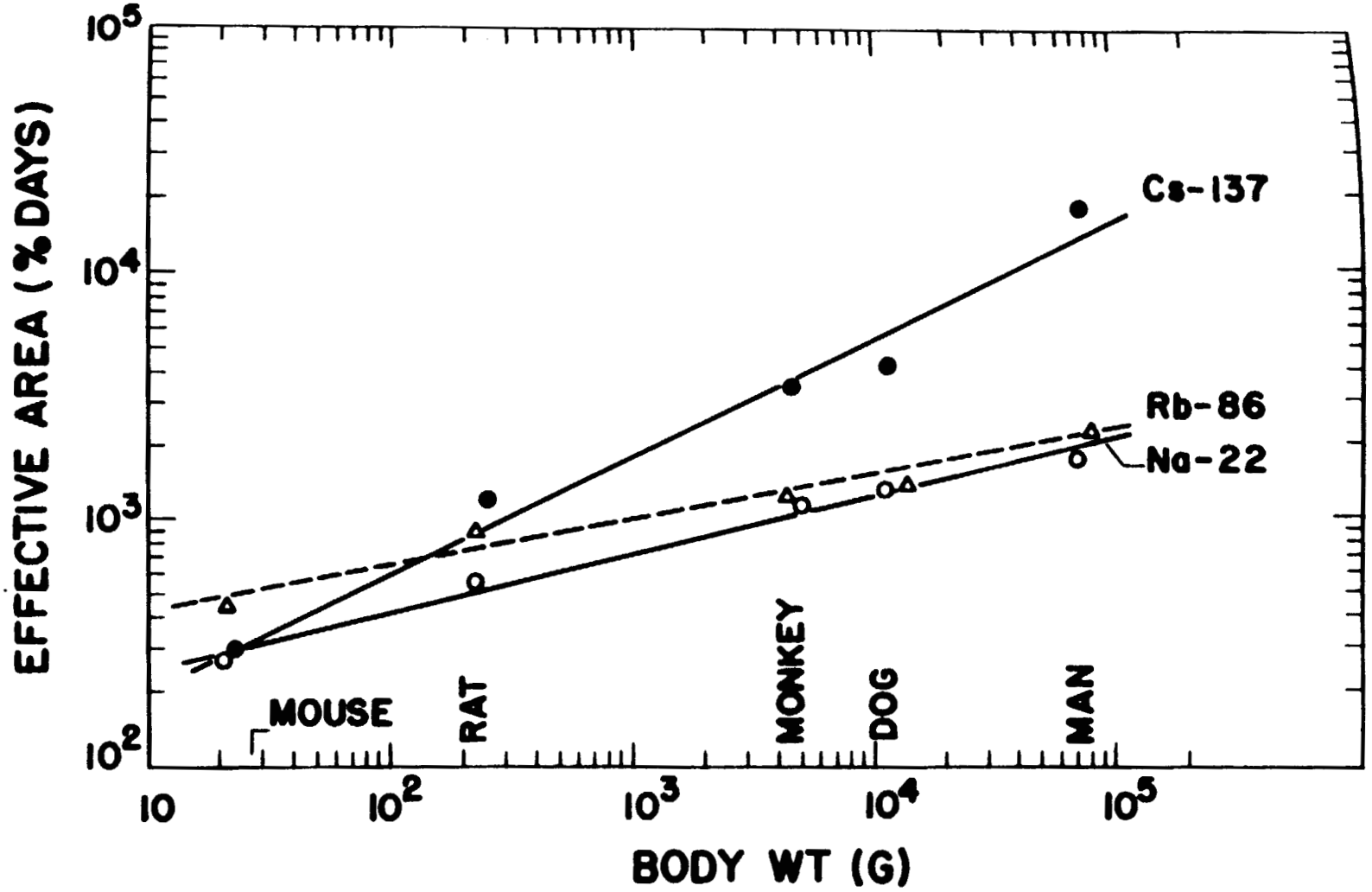


Fig. 4. Interspecies correlation between radioisotope retention (effective area under the curve) and body weight.

Metabolism of Zinc^{65} in Mammals (C. R. Richmond, J. E. Furchner, and G. A. Trafton)

Introduction

Zinc is known to be an essential trace element. It functions as a prosthetic group in enzymes concerned with proteolysis and others concerned with gaseous and cellular respiration. Zinc may also participate in erythropoiesis and in the functioning of myelogenous bone marrow.

Although Zn^{65} is not a product of nuclear fission, it is produced via neutron activation in power reactors and nuclear detonations. Consequently, small amounts of Zn^{65} have been detected in the flora and fauna of several areas, in fallout, and in commercial food supplies. Zinc^{65} has also been identified in several Marshallese subjects and some people in the vicinity of the Hanford works. Gamma ray spectra obtained from several cyclotron workers also show the presence of Zn^{65}. Presumably, the radiozinc is produced in the cyclotron as a contaminant by the $Cu^{65}(d,2n)Zn^{65}$ reaction.

The presence of Zn^{65} in food and people as a contaminant from nuclear weapons tests, reactor effluents, and nuclear research operations motivated this study.

Methods and Results

Absorption, excretion, and retention of Zn^{65} were studie

in 4 species of mammals by whole body radioassay techniques. Biological retention functions obtained by these procedures following single acute oral doses (≥ 1 μc) of $Zn^{65}Cl_2$ can be expressed as composite exponential functions of the form

$$R_t = \sum_i^n \left[a_i e^{-k_i t} \right].$$

Retention curves for mice, rats, dogs, and man (2 subjects), calculated from the equations in Table 1, are shown with the measured retention values in Fig. 1. These equations were determined by a computer method which gave the best fit to the experimental data. Equations 4 and 5 (Table 1) represent data obtained from 2 normal men for periods of 12 and 15 months, respectively. Changes in whole body activity will be followed until low activities preclude further measurements.

Table 2 gives the parameters of the effective retention function for mice and the area under each exponential function contributing to the over-all loss from t_o to t_∞. The area is proportional to the number of disintegrating atoms and is, therefore, related to dose. Table 2 also shows that if equilibrium conditions were established during chronic exposure, about 52 per cent of the Zn^{65} atoms in the mouse would be lost with an effective half-time of ~ 91 days. A comparison of the total effective areas under the retention functions for various species is shown in Table 3. Most of the area under

TABLE 1. Whole Body Biological Retention Functions of Zn^{65} following Oral Administration to Four Mammalian Species

Mice (12)	$^{*}R_t = 86.82 \exp(-2.3421t) + 11.19 \exp(-0.0572t) + 1.84 \exp(-0.0048t)$
Rats (6)	$R_t = 77.01 \exp(-1.4672t) + 13.83 \exp(-0.0447t) + 8.84 \exp(-0.0063t)$
Dogs (4)	$R_t = 51.63 \exp(-1.2208t) + 10.06 \exp(-0.0743t) + 38.74 \exp(-0.0045t)$
[redacted]**	$R_t = 8.89 \exp(-0.7877t) + 22.80 \exp(-0.0316t) + 68.57 \exp(-0.0016t)$
[redacted]	$R_t = 38.80 \exp(-2.4713t) + 11.37 \exp(-0.1428t) + 49.84 \exp(-0.0016t)$

*t is expressed in days.

**LC indicates laboratory case.

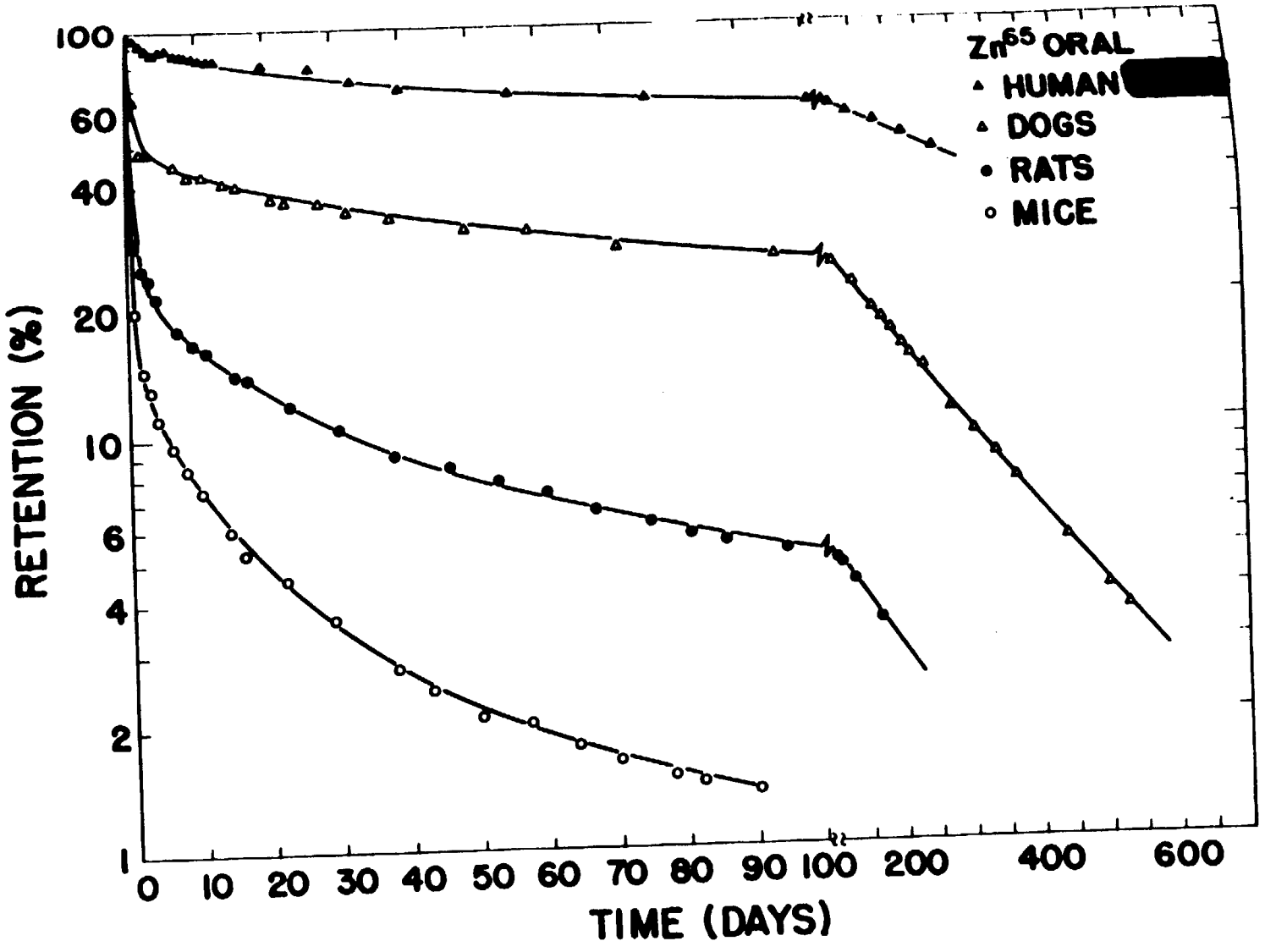


Fig. 1. Retention of orally administered Zn^{65} by mice, rats, dogs, and man.

TABLE 2. "Effective" Areas under Components of Composite Retention Function* of Zn^{65} Administered Orally to Mice

Component	a	T	k + λ	Area** (% days)	% of Total Area
1	86.82	0.30	2.3449	37.5	8.09
2	11.19	11.55	0.0600	186.4	40.22
3	1.84	91.18	0.0076	239.6	51.69

*Retention function $R_t = \sum_i^n \left[a_i e^{-k_i t} \cdot e^{-\lambda t} \right]$.

**Effective area $A = \sum_i^n \left[\frac{a_i}{k_i + \lambda} \right]$ = 463.5 per cent days.

TABLE 3. Total and Percentage "Effective" Areas under the Composite Retention Functions of Zn^{65} Administered Orally to Four Mammalian Species

Species	Component and Per Cent of Total Area			Total Area (% days)
	1	2	3	
Mouse	8.09	40.22	51.69	464
Rat	4.01	22.23	73.77	1308
Dog	0.77	2.38	96.84	5465
[redacted]	0.07	4.10	95.83	16131
[redacted]	0.14	0.69	99.17	11337

*LC indicates laboratory case.

each retention function is related to the component with the longest half-time. The difference in effective area between mice and man (25 to 35 fold) is the result of the greater gastrointestinal uptake and the increased retention time of the latter. Figure 1 shows the whole body retention functions for the 4 species and suggests the possibility of heterogonic relationships between total effective area, intestinal uptake, and size of the species.

Figure 2 shows the relationship between body weight of the species and uptake (amount retained at 2 days), as well as the relationship of body weight to the effective area of the retention function. An interspecies correlation between these parameters and body weight seems to exist, and the effective area (Y) in terms of the body weight (W) is given by the expression

$$Y = 146.0\ X^{0.3977}.$$

Although a log-log relationship is insensitive, human metabol parameters derived in this way should be preferable to experi mental animal data for calculation of maximum permissible lev of radioisotopes.

Tissue distribution studies in rats showed that the lon component of the retention function represented loss of Zn^{65} from bone and hair. However, because the biologically effec

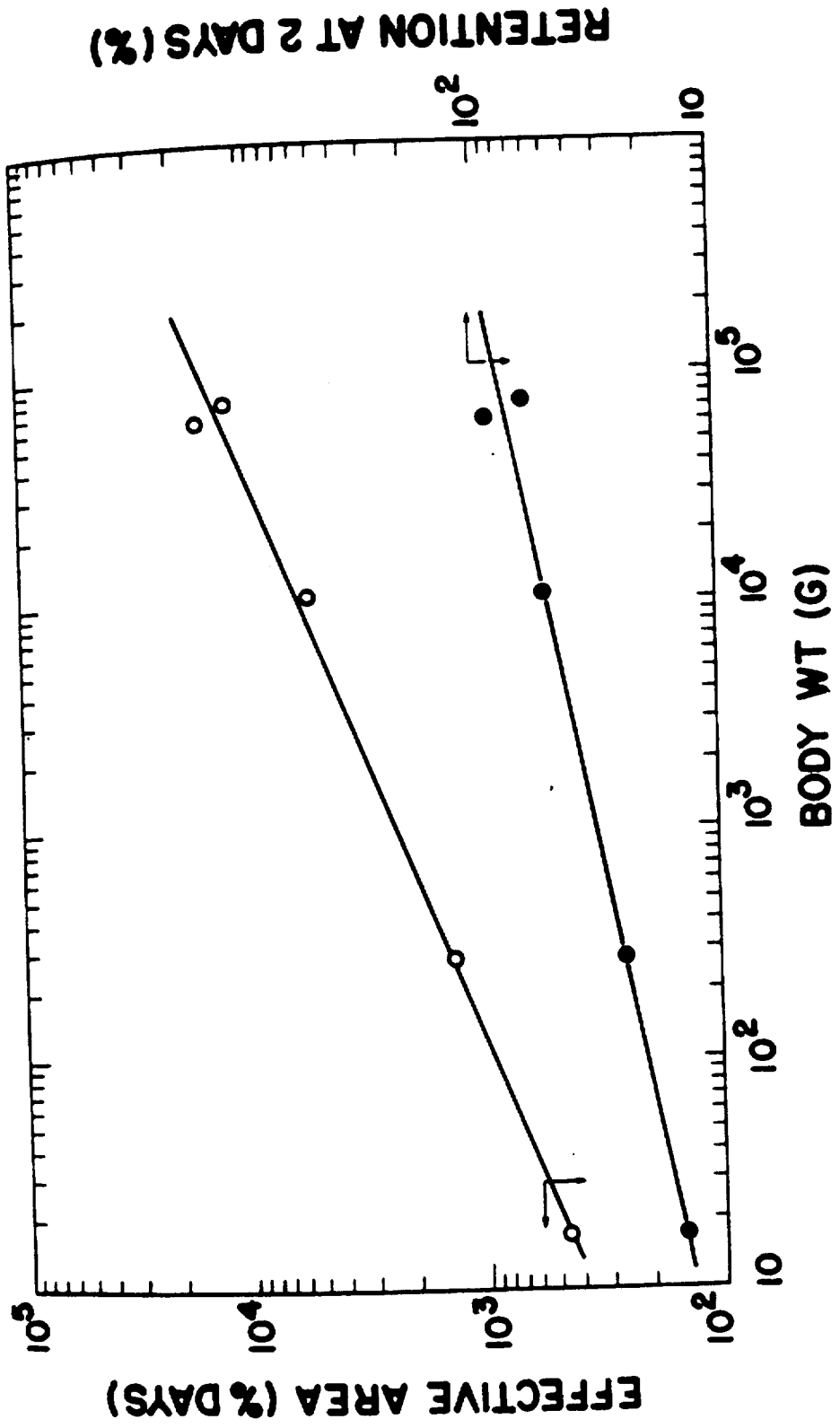


Fig. 2. Interspecies relationship between uptake and retention of Zn^{65} and body weight.

energy of Zn^{65} is hard gamma radiation, the whole body may be considered as the critical organ. A comparison of presently recommended maximum permissible concentrations of Zn^{65} in water, $(MPC)_w$, with those calculated from the present data for man is shown in Table 4. The two values for [redacted] resulted from using the parameters from the long component only (c) and those for all 3 components (d). The ratio of c to d ($\sim$1) is the same as the ratio of the area under the long component to the total area (Table 3, [redacted].

TABLE 4. Maximum Permissible Concentrations of Zn^{65} in Water $(MPC)_w$[a]

	T_b (days)	T (days)	f_1	f_w ($f_1 f_2$)	$(MPC)_w$
ICRP (1952)	23	21	0.10	0.10	2.6×10^{-2}
ICRP (1959)	933[b]	194	0.10	0.10	2.8×10^{-3}
[redacted][d]	445	157		0.50	7.0×10^{-4}
[redacted]				1.00[c]	7.0×10^{-4}
[redacted]	445	157		0.69	5.1×10^{-4}

[a] $(MPC)_w = \dfrac{9.2 \times 10^{-4}\ q\ f_2}{T\ f_w(1 - e^{-0.693t/T_)}}$. Critical organ = whole body; q = 60 μc.

[b] Calculated value.

[c] Denominator of $(MPC)_w$ equation = $f_w\left[\sum_i^n a_i T_i \left(1 - e^{\frac{-0.693t_i}{T_i}}\right)\right]$.

[d] LC indicates laboratory case.

Metabolism of Zirconium^{95} and Ruthenium^{106} in Mammals (C. R. Richmond, J. E. Furchner, and G. A. Trafton)

Introduction

Zirconium^{95}, Ru^{106}, and Ce^{144} are radionuclides formed in relatively high yield in the nuclear fission process. Zirconium^{95}/Nb^{95} and Ru^{106}/Rh^{106} have been identified in animal tissues and foods by gamma ray spectrometry. Cerium^{144}/Pr^{144} has been found in the rumen contents of animals and possibly in some foods. Although they are known to be poorly absorbed, the increasing presence of these relatively short-lived radionuclides in the environment suggests the need for further investigation of their absorption, retention, and excretion by laboratory animals and man.

Methods and Results

A multiple tracer experiment using Zr^{95}/Nb^{95}, Ru^{106}/Rh^{106}, and Ce^{144}/Pr^{144} in rats provided preliminary information on gastrointestinal absorption. About 3.9 per cent of the zirconium, 1.5 per cent of the ruthenium, and about 0.6 per cent of the cerium remained in the animals on the second day following oral intubation. These numbers probably reflect some differences in excretion rates as well as gastrointestinal absorption; however, the former is relatively unimportant in determining the observed value because of the

short period of observation. When administered orally, such small fractions of these substances are absorbed from the gastrointestinal tract that the retained activity soon falls below the detectable limits of most measuring techniques. For this reason, their retention in rats and mice was studied after intraperitoneal administration of the radionuclides. We have found that whole body retention functions after oral and intraperitoneal administration of Zn^{65}, Sr^{85}, and Ba^{133} are the same if corrected for the quantity not absorbed from the gastrointestinal tract.

Figure 1 shows whole body retention data for ~140 days in 2 groups of rats injected intraperitoneally with tracer doses of Ru^{106}/Rh^{106} and Zr^{95}/Nb^{95} and assayed periodically in an in vivo scintillation counter. It appears that the half-time of the long component of these materials will be considerably greater than indicated in the National Bureau of Standards Handbook No. 69. Data are being or will be collected for mice, rats, dogs, monkeys, and possibly man following acute oral administration for purposes of interspecies comparisons.

Daughter products from Ce^{144} and Ru^{106} do not complicate retention studies because of their short half-lives. Because Nb^{95} has a half-life about half that of its parent (Zr^{95}), the retention and excretion of Nb^{95} are also being studied.

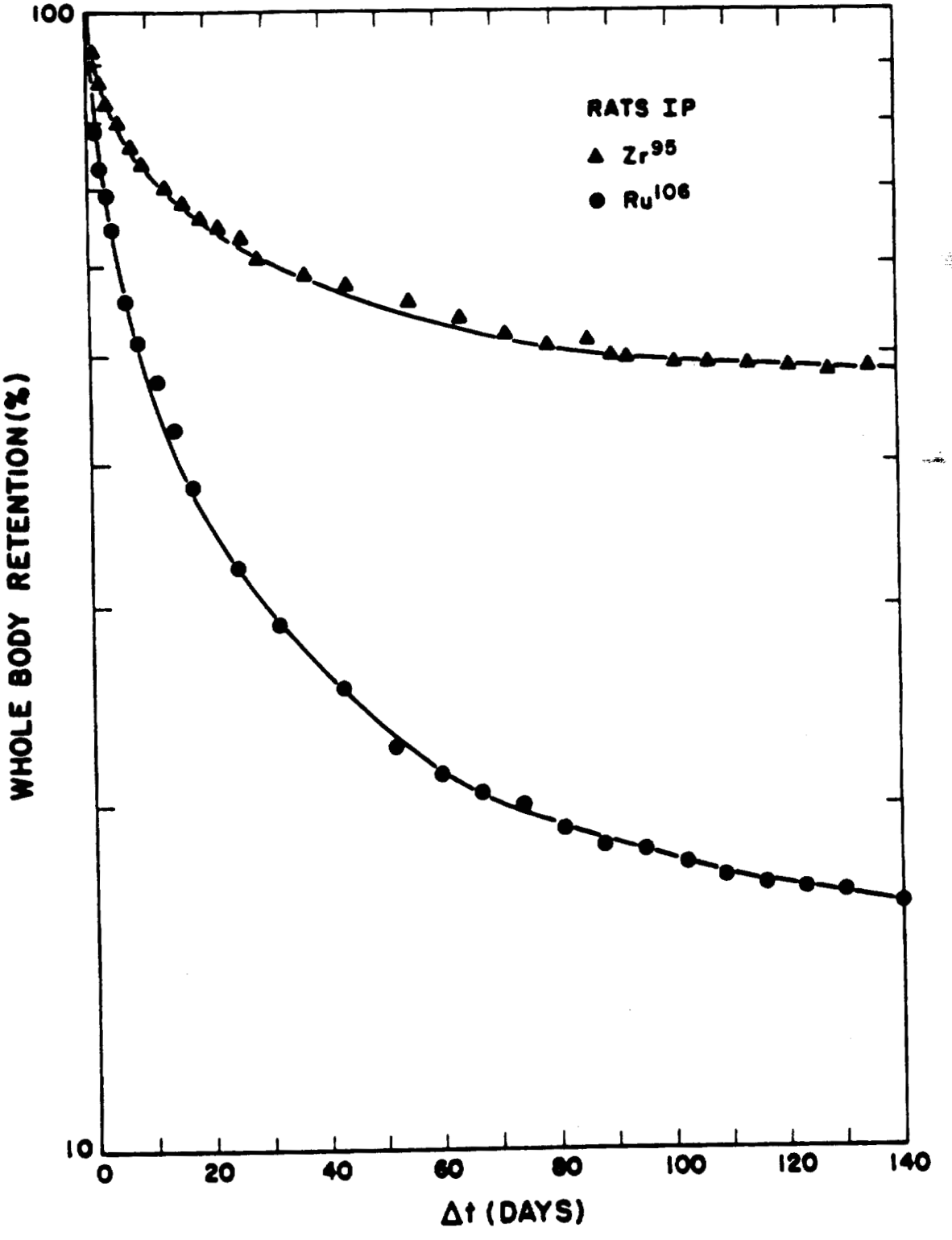


Fig. 1. Whole body retention of intraperitoneally administered Ru^{106}/Rh^{106} and Zr^{95}/Nb^{95} by rats.

A newly designed small animal liquid scintillation counter, LASAC-III (Los Alamos Small Animal Counter III), will be more sensitive to relatively weak gamma radiations than the counters currently in use. Whole body retention of Ce^{144}/Pr^{144} and other radionuclides with low energy gamma radiations will be investigated in small mammals using this equipment.

Volume and Turnover of Body Water in Various Mammalian Species Using Tritiated Water as a Tracer (C. R. Richmond, T. T. Trujillo, and W. H. Langham)

Introduction

The kinetics of body water retention were studied in 7 mammalian species as part of a general program to investigate interspecies correlations in the metabolism of radionuclides by mammals.

Methods and Results

Tritiated water was used as the tracer. Table 1 gives the details of the individual experiments. To determine total loss (or replacement) of body water per unit time, one must know the size and turnover time of the body water pool. These parameters can be determined from the Y-intercept and the half-time of the regression function which relates logarithm of HTO concentration in body water to time after administration. Table 1 also summarizes the results for half-time and body water for the various species. The retention process for all species (throughout the period of study) was best described by a single exponential function. Data for Dipodomys deserti (Kangaroo rat) are included as these animals are atypical as regards water metabolism. For those cases where comparisons can be made, individual values in

TABLE 1. Volume and Turnover of Body Water in Seven Mammalian Species

Species	Sex	Wt. (g)	Dose (mc)	Route of Administration	Body Water (% of Body Wt.)	Half-time (days)	Water Loss[a] (ml/day)
Mouse	F	21.4 (24)[c]	0.708	I. P.	58.49 ± 3.97[b] (24)	1.13 ± 0.14 (12)	7.22 ± 1.15 (12)
Rat	M	298 (12)	4.329	I. P.	59.61 ± 4.04 (11)	3.53 ± 0.40 (12)	34.54 ± 4.90 (11)
Dipodomys[d]	M,F	93 (20)	0.850	I. P.	62.20 ± 2.35 (20)	11.82 ± 2.96 (10)	3.75 ± 0.95 (10)
Rabbit	F	3159 (4)	7.100	I. P.	58.35 ± 5.31 (4)	3.87 ± 0.21 (4)	338 ± 62 (4)
Dog	M	10,582 (5)	1.270	Oral	65.95 ± 1.42 (5)	5.14 ± 0.18 (5)	946 ± 124 (5)
Man	M,F	67,302 (5)	2.000	Oral	55.34 ± 5.31 (5)	9.46 ± 0.88 (5)	2720 ± 488 (5)
Horse	M	398,533 (3)	142.1	I. V.	65.71 ± 0.72 (3)	8.41 ± 0.53 (3)	21,722 ± 3247 (3)

[a] From the derivative of $V_t = V_o e^{-kt}$, when V_o is the volume of exchangeable body water (ml), t is time zero, and k is the rate constant of the exponential function (fractional change per day).

[b] Mean ± standard deviation.

[c] Number in parentheses is the number of individual animals composing the average.

[d] Kangaroo rats. Maintained in laboratory during experiments on dry pearled barley alone. No other sources of exogenous water were available.

Table 1 (columns 6, 7, and 8) agree reasonably well with existing data.

Figure 1 shows an allometric relation between daily body water loss and body weight. The coefficient of correlation for the regression line is 0.996 and the 95 per cent confidence limits are shown for the plotted mean values. Kangaroo rats do not fall within the 95 per cent confidence limits of the regression line. The rate of body water turnover is about one-third that expected from the regression line. A mean half-time of ~12 days for body water retention (Table 1) is indicative of the high degree of water conservation practiced by these animals. Adolph (1) reported a regression coefficien of 0.88 for an interspecific comparison of daily water intake as a function of body weight.

Although the volume and turnover of body water may vary markedly among species, these factors can be used to determine the volume of daily loss which is related interspecifically to body weight. One should be able to estimate fairly accurately the turnover time of body water for many mammalian species by using this equation and assuming the body water to be 60 per cent of the body weight. For example, the turnover time for a 10-ton elephant would be about 25 days. This represents a biological half-time of about 17 days. Figure 2 shows a plot of the log of total body water of the various

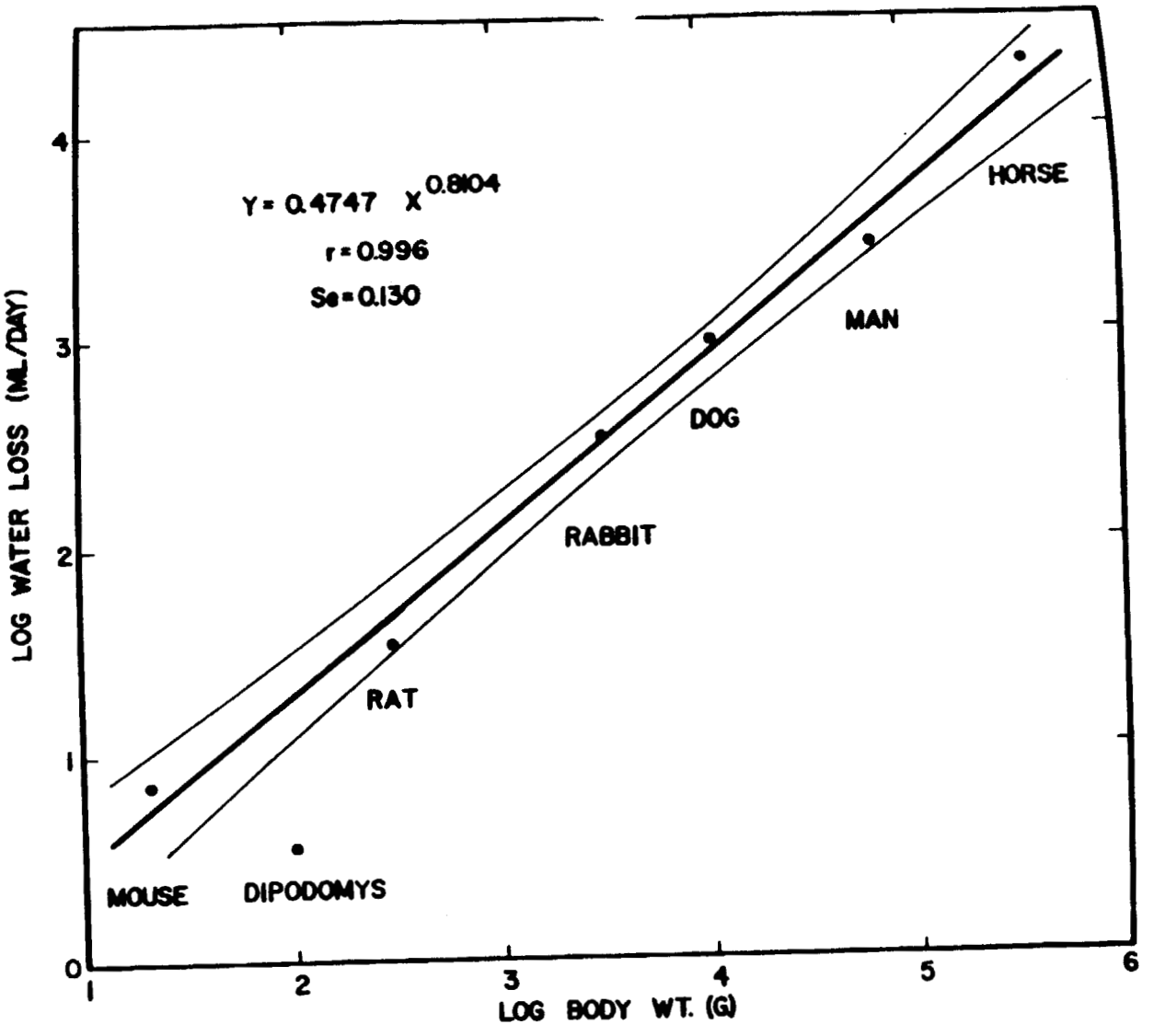


Fig. 1. Interspecies relationship between daily body water loss and body weight.

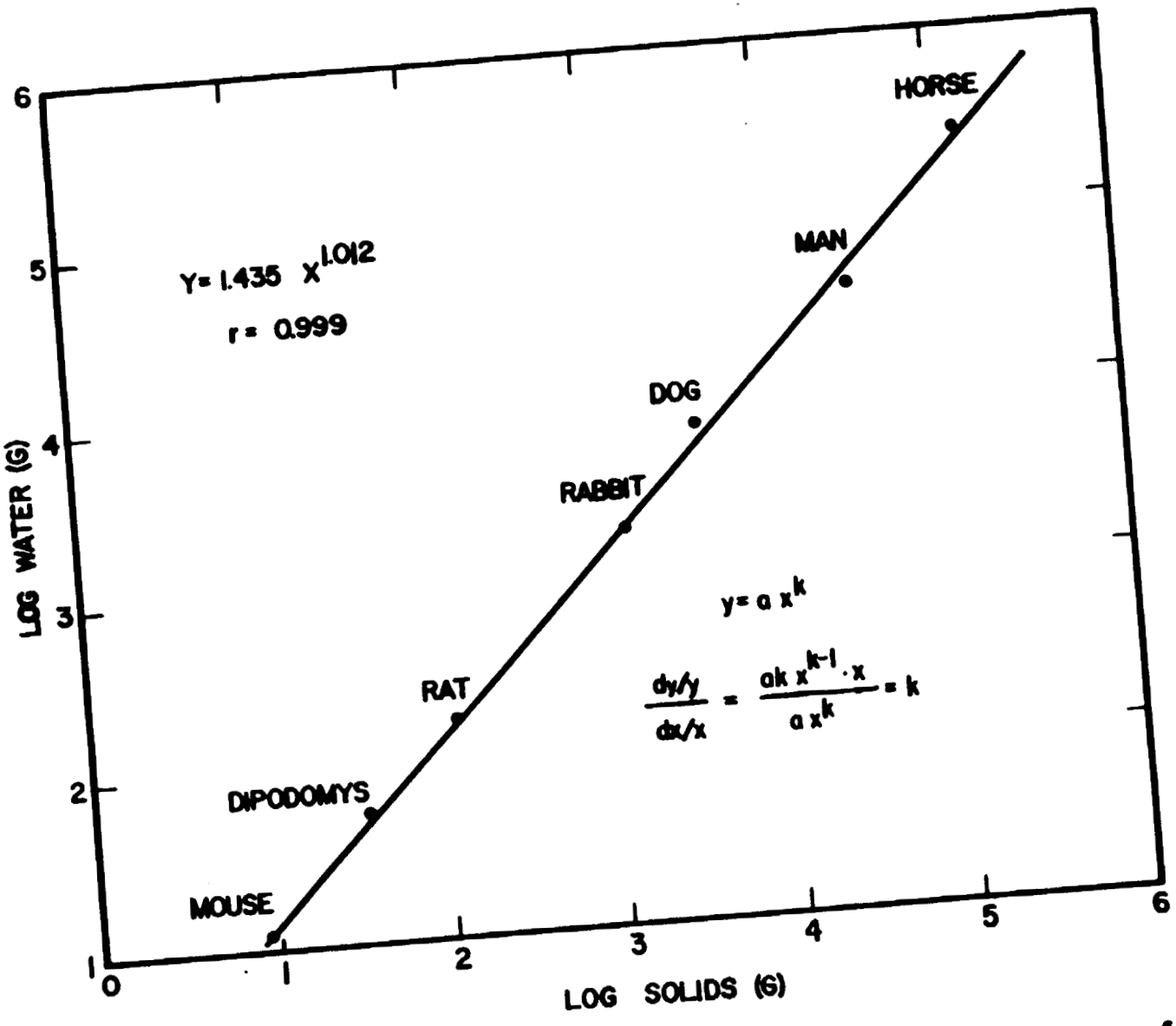


Fig. 2. Interspecies relationship between total body water and mass of body solids.

species as a function of the log of the body solids. These data merely show that the ratio of water to solids is a constant for the 7 species and show that the degree of hydration of the tissues of the Kangaroo rat is quite normal. This emphasizes the known fact that the unusual ability of this species to live in the desert involves a special adaptation of the kidneys for the conservation of water.

Reference

(1) E. F. Adolph, Science 109, 579 (1949).

BIOCHEMISTRY SECTION PUBLICATIONS

(1) R. G. Gould, The Relationship between Thyroid Hormones and Cholesterol Biosynthesis and Turnover. In: Hormones and Atherosclerosis, G. Pincus, ed., Academic Press (1959), pp. 75-88.

(2) R. G. Gould, E. H. Lilly, and V. E. Mitchell, Effects of MER-29 on Tissue and Plasma Cholesterol Concentrations and on Hepatic Cholesterol Synthesis from Mevalonic Acid, Circulation 20, 965 (1959).

(3) R. G. Gould, R. J. Jones, and R. W. Wissler, Lability of Cholesterol in Human Atherosclerotic Plaques, Circulation 20, 967 (1959). Also in: Semiannual Report to the U. S. Atomic Energy Commission, Argonne Cancer Research Hospital (September 1959), p. 62.

(4) W. H. Langham, Physiology and Toxicology of Plutonium-239 and Its Industrial Medical Control, Health Phys. 2, 172 (1959).

(5) W. H. Langham, Physiology and Toxicology of Plutonium^{239}. In: 1959 Nuclear Congress, Proceedings of the Seventh Hot Laboratories and Equipment Conference, April 7-9 (1959), pp. 256-274.

MANUSCRIPTS SUBMITTED AND ACCEPTED

(1) H. Foreman, The Pharmacology of Some Useful Chelating Agents, to be published in Metal-Binding in Medicine. Proceedings of a Symposium sponsored by the Hahnemann Medical College and Hospital, Philadelphia (May 6-8, 1959).

(2) H. Foreman, Application of Chelating Agents for Hastening Excretion of Radioelements, to be published in Metal-Binding in Medicine. Proceedings of a Symposium sponsored by the Hahnemann Medical College and Hospital, Philadelphia (May 6-8, 1959).

(3) R. G. Gould, Effects of MER-29 on Sterol Concentration in Rat Tissues and on Cholesterol Biosynthesis in vivo and in vitro from Mevalonic Acid-2-C^{14}, to be published in book form.

(4) W. H. Langham, Radioisotopes - Absorption and Methods of Elimination: Relative Significance of Portals of Entry, to be published in Proceedings of the Symposium on Radioisotopes in the Biosphere, held at the University of Minnesota, Minneapolis (October 19-23, 1959).

(5) D. F. Petersen and A. Murray III, Simple Fluorescent Intensification Screen for Ultraviolet Scanner Cameras, Anal. Chem.

(6) C. R. Richmond, T. T. Trujillo, and D. W. Martin, Determination of Volume and Turnover of Body Water in Dipodomys Deserti with Tritiated Water, Proc. Soc. Exptl. Biol. Med.

CHAPTER 3

LOW-LEVEL COUNTING SECTION

Monitoring of Milk for K^{40}, Cs^{137}, and Ba^{140}/La^{140}

(B. Clinton, J. Allen, and E. C. Anderson)

Introduction

A project to monitor systematically the U. S. powdered milk supply for Cs^{137}, K^{40}, and Ba^{140}/La^{140} was begun in 1956. During 1957-1958, additional collection stations were included to give more complete coverage and to provide information on specific questions. The network of collection stations as of the present time is shown in Fig. 1. Tabulations of the 1959 data are being published quarterly in the New York Health and Safety Laboratory's Strontium Program reports (1-4). A complete tabulation of 1958 data was published in a Los Alamos Scientific Laboratory report (5), which included a description of the method of measurement. Summaries and interpretations of the 1956 and 1957 results were published in two Science articles (6,7). A general

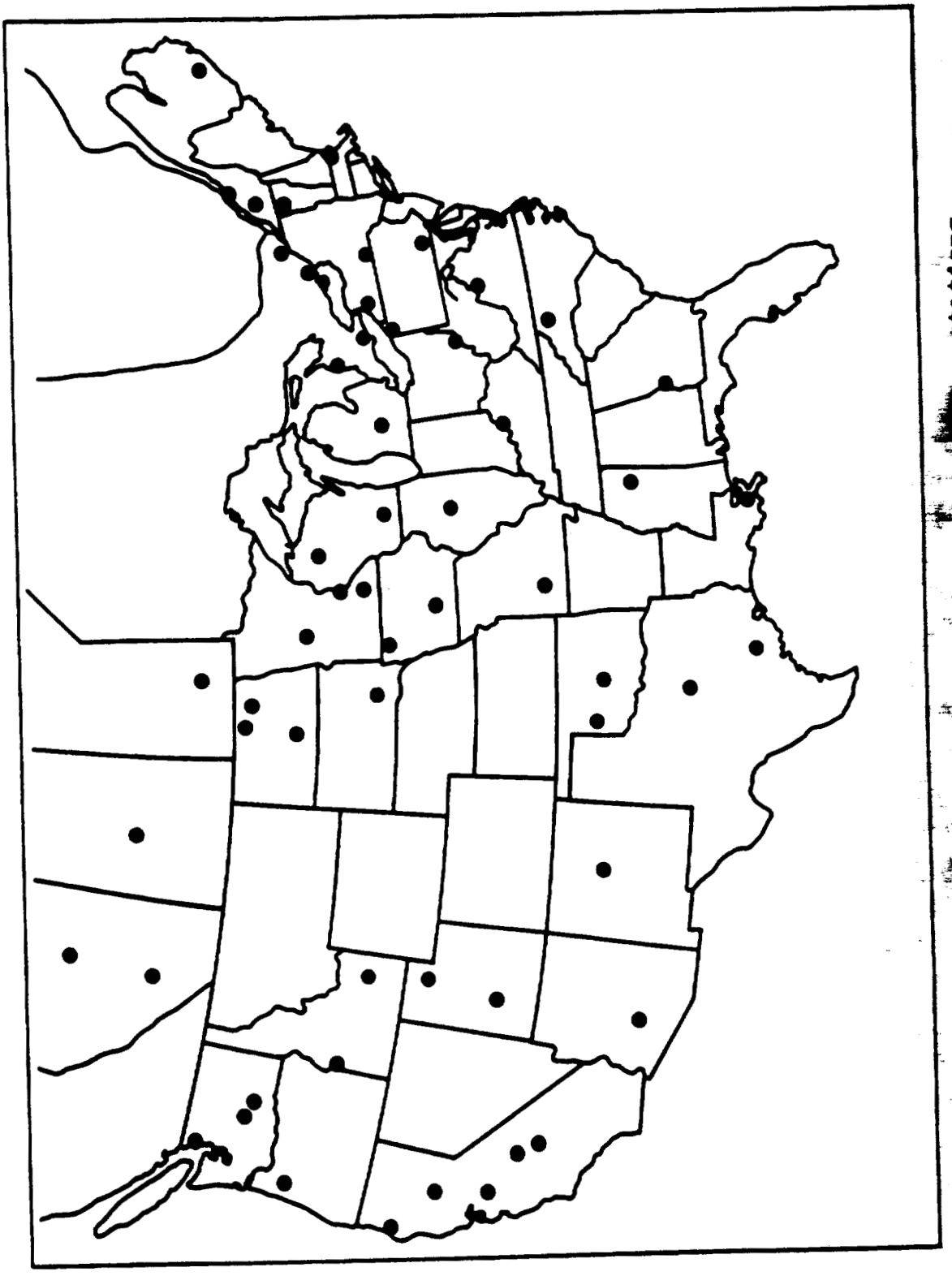


Fig. 1. Los Alamos Scientific Laboratory's milk [illegible] stations.

summary and interpretation of 1958 results have not been published, although pertinent conclusions drawn therefrom have been made known through discussions and correspondence with interested people and agencies. In the interest of providing continuity, the present report includes discussion of results obtained prior to the present report period and some review of past reports and of the 1958 results.

Results and Discussion

An earlier interpretation of 1957 Cs^{137} levels in U. S. powdered milk as a function of rainfall (7) showed that the country could be divided into two well defined regions. One (in which the Cs^{137} per inch of rainfall was high) included the western and northwestern and far northern states, and the other (with low Cs^{137} values) included the southern and eastern states. In this analysis of the data, all samples showing the presence of Ba^{140}/La^{140} were eliminated. Figure 2 shows a re-evaluation of the 1957 data on the basis of total precipitation, including all samples. In this case, of course, the averages are somewhat higher. Division of the country grossly into two more or less well defined regions still persists. Values in some of the western states and especially in California, however, seem to fall into an intermediate region. High tropospheric fallout anomalies

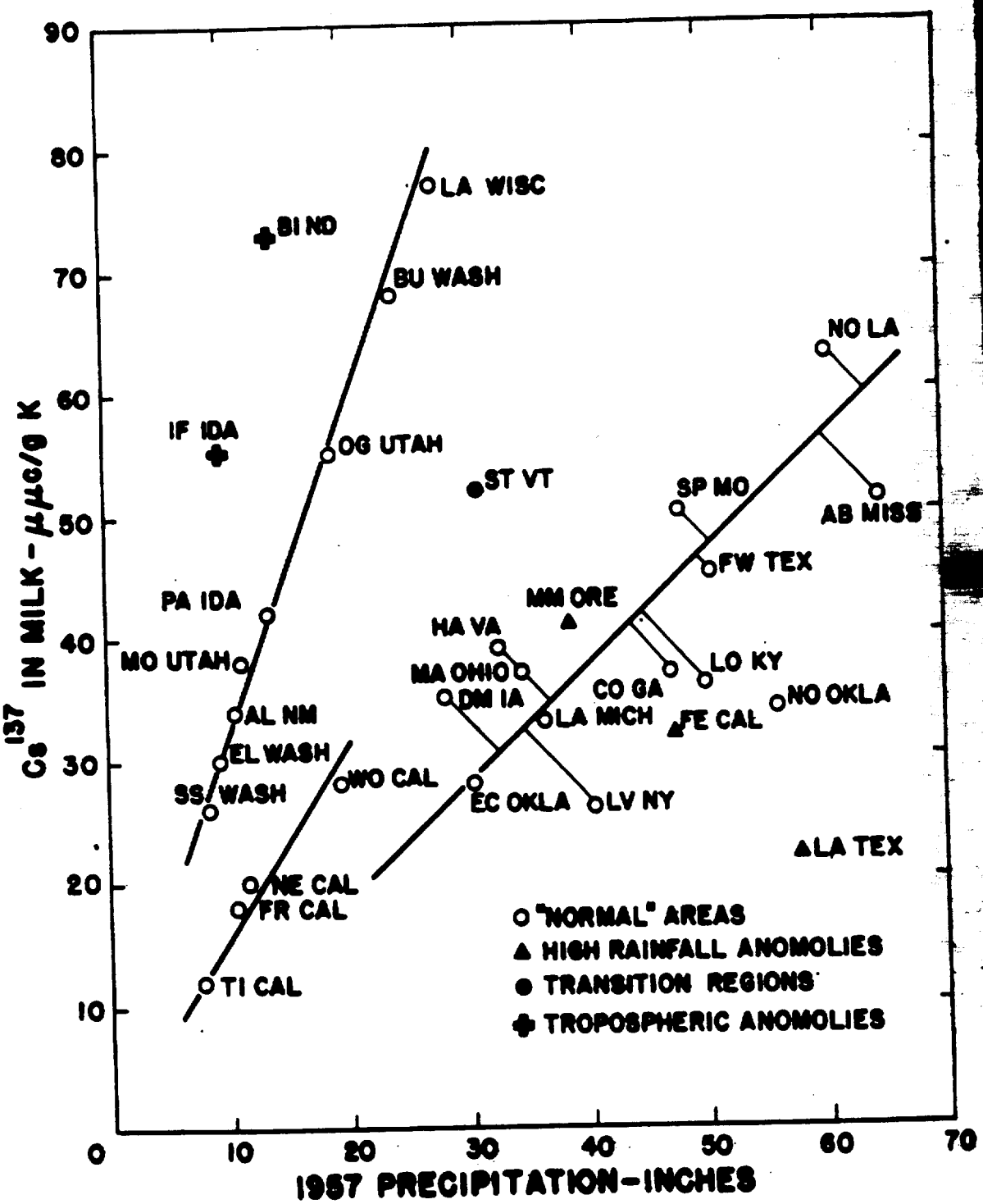
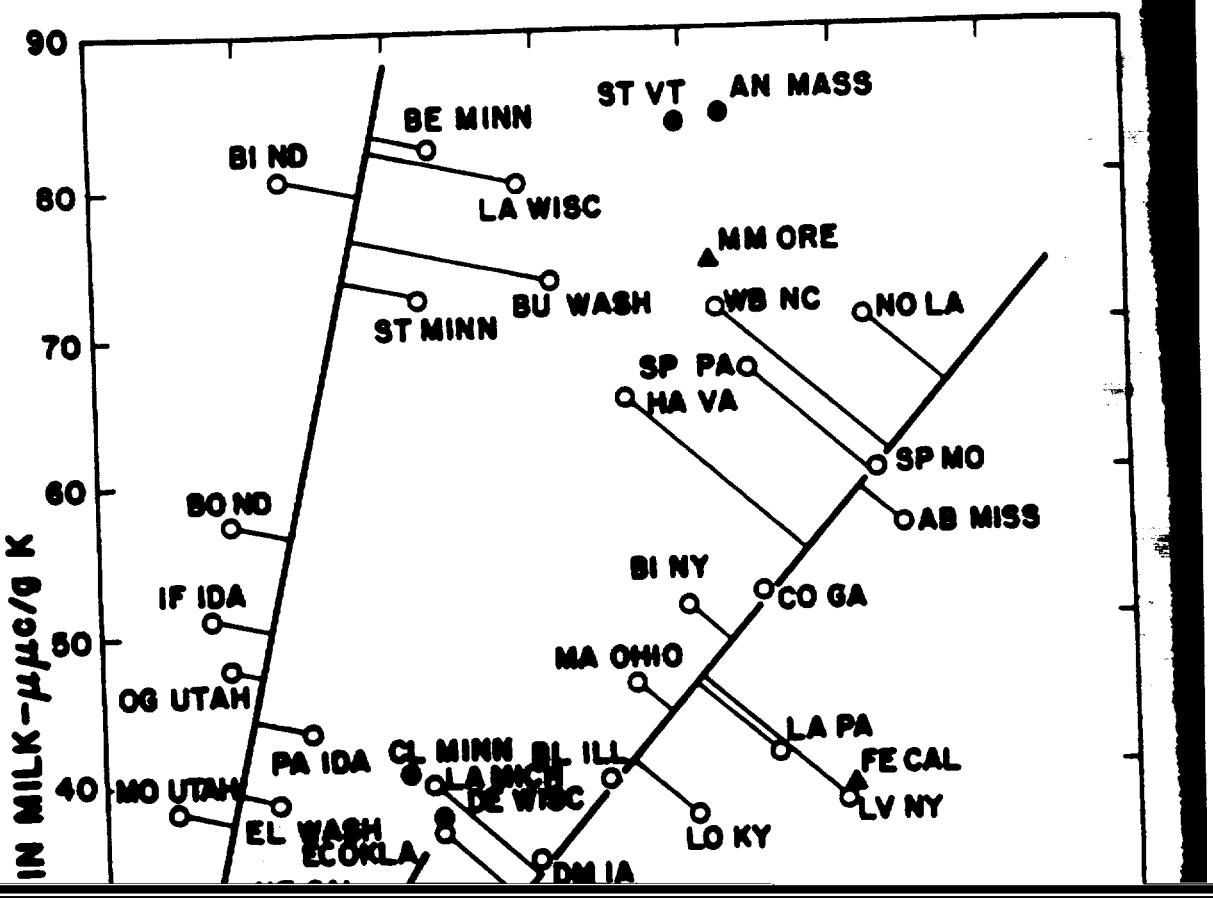


Fig. 2. Cesium^{137} in milk versus rainfall (1957).

are shown by Bismarck, North Dakota, and Idaho Falls, Idaho. In these cases, prevailing winds are from the general direction of the Nevada Test Site favoring high tropospheric fallout. High rainfall anomalies also occur (McMinnville, Oregon; Fernbridge, California; and LaGrange, Texas). These are high local rainfall areas, where total precipitation is considerably higher than the average for the general region in which they occur. This results in relatively lower Cs^{137} activity per inch of precipitation. Locations or areas near the diffuse or ill-defined borders of the two general regions may be expected to show up as transition anomalies. Only one such anomaly (St. Albans, Vermont) is indicated by the 1957 data.

Figure 3 shows a corresponding treatment of the 1958 milk data. The general picture remains the same as that for the 1957 results, with perhaps a somewhat greater scatter. The greater scatter may have resulted from a number of factors. The 1958 bomb testing programs were unusually large and spread out over a greater period, and for the first time large amounts of debris were injected into the environment at far northerly latitudes. New milk sampling stations also were introduced to represent possible transition anomalies. Many of the additional sites included during 1958 were chosen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to lie geographically along the boundary between the two regions defined by the 1957 studies (including Sibley, Iowa; Claremont, Minnesota; Deerfield, Wisconsin; and Andover, Massachusetts), and these indeed show an intermediate ratio of Cs^{137} to precipitation. Other new points fall into their respective main regions: Bertha and Stillwater, Minnesota, and Bottineau, North Dakota, in the region with rainfall of high specific activity, and Bloomington, Illinois; Lancaster and Springboro, Pennsylvania; Binghamton, New York; and Wilkesboro, North Carolina, in the region with lower specific activity rainfall. The high rainfall anomalies remain as before. No tropospheric anomalies were observed, consistent with the much lower Ba^{140}/La^{140} levels during this year, and perhaps consistent with the fact that the 1958 Nevada tests were held later in the year and under conditions that minimized tropospheric contamination.

A comparison between the average Cs^{137} milk levels for 1957 and 1958 is given in Fig. 4. Many regions showed little or no change, while others appeared to have increased 30 to 60 per cent. Only four out of 30 points showed a slight decrease. No conclusions concerning direct versus indirect

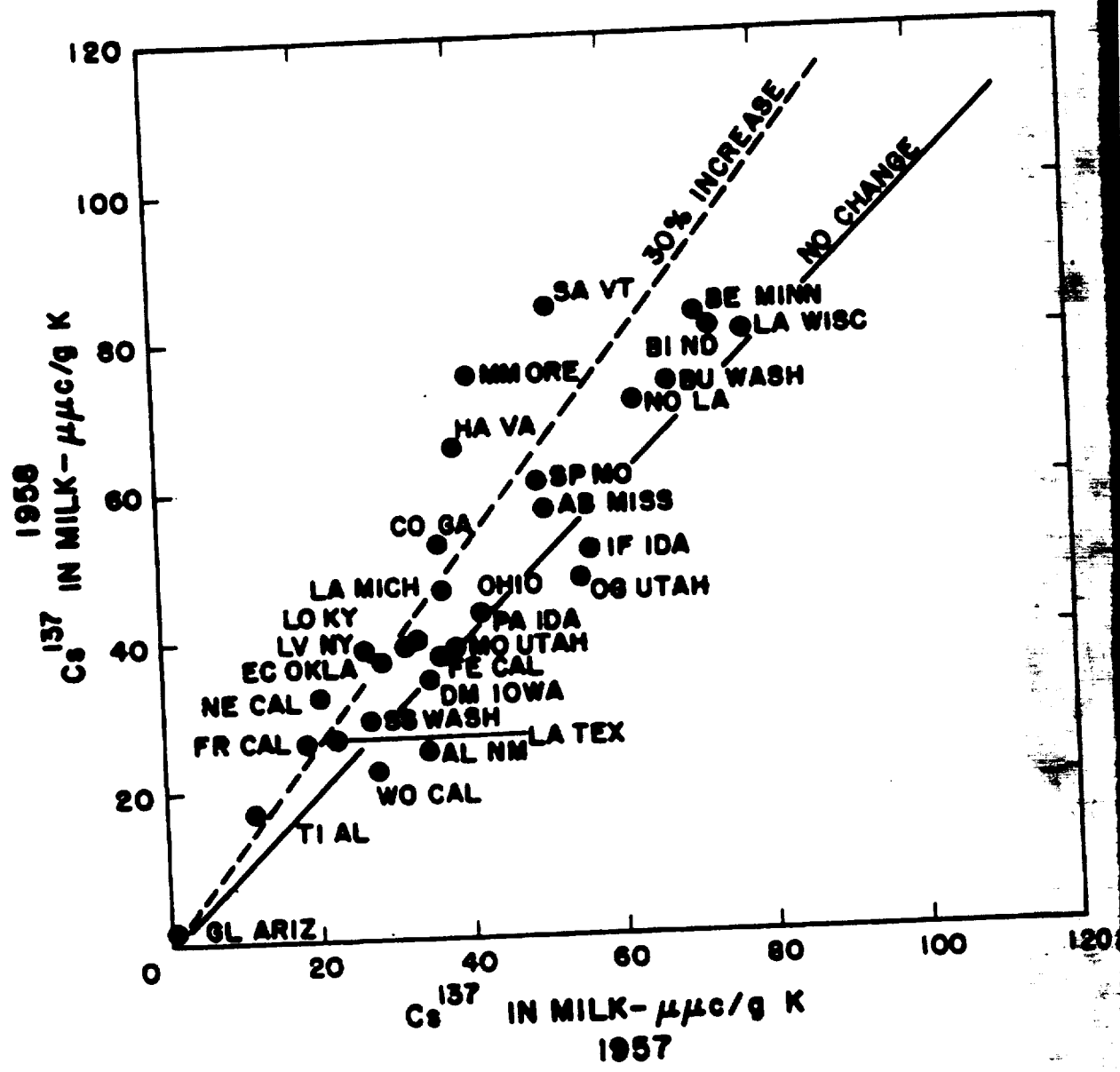

Fig. 4. Cesium^{137} in milk (1958) versus 1957.

ecological uptake can be derived from this comparison, since both the integrated level and rate of fallout increased during 1958 by about 40 per cent above the 1957 level. Clearly, many of the milk samples failed to rise as much as expected on either basis.

Figure 5 shows an analogous comparison of the Cs^{137} in people for 1957 and 1958. While there is considerable scatter, the results are not inconsistent with an increase of 30 per cent or so, as in the case of the milk samples. The four New Mexico points refer to quarterly, rather than yearly, averages.

Figure 6 gives the relationship between the Cs^{137} in people with that in milk from the same general area for the year 1958. Again, the New Mexico averages are by quarters. Some of the scatter is certainly due to failure of the milk sources to correspond with the population sample or (as may be the case with Kansas, Missouri, the Southeast, and Oregon) too few measurements on people to provide a representative sample. There is no systematic program for measuring Cs^{137} levels in the population. Sampling consists only of the occasional Los Alamos visitor who agrees to being measured. The slope of the curve again suggests a discrimination factor of about 2 for cesium to potassium on going from milk to man. As concluded from the 1957 results, roughly half

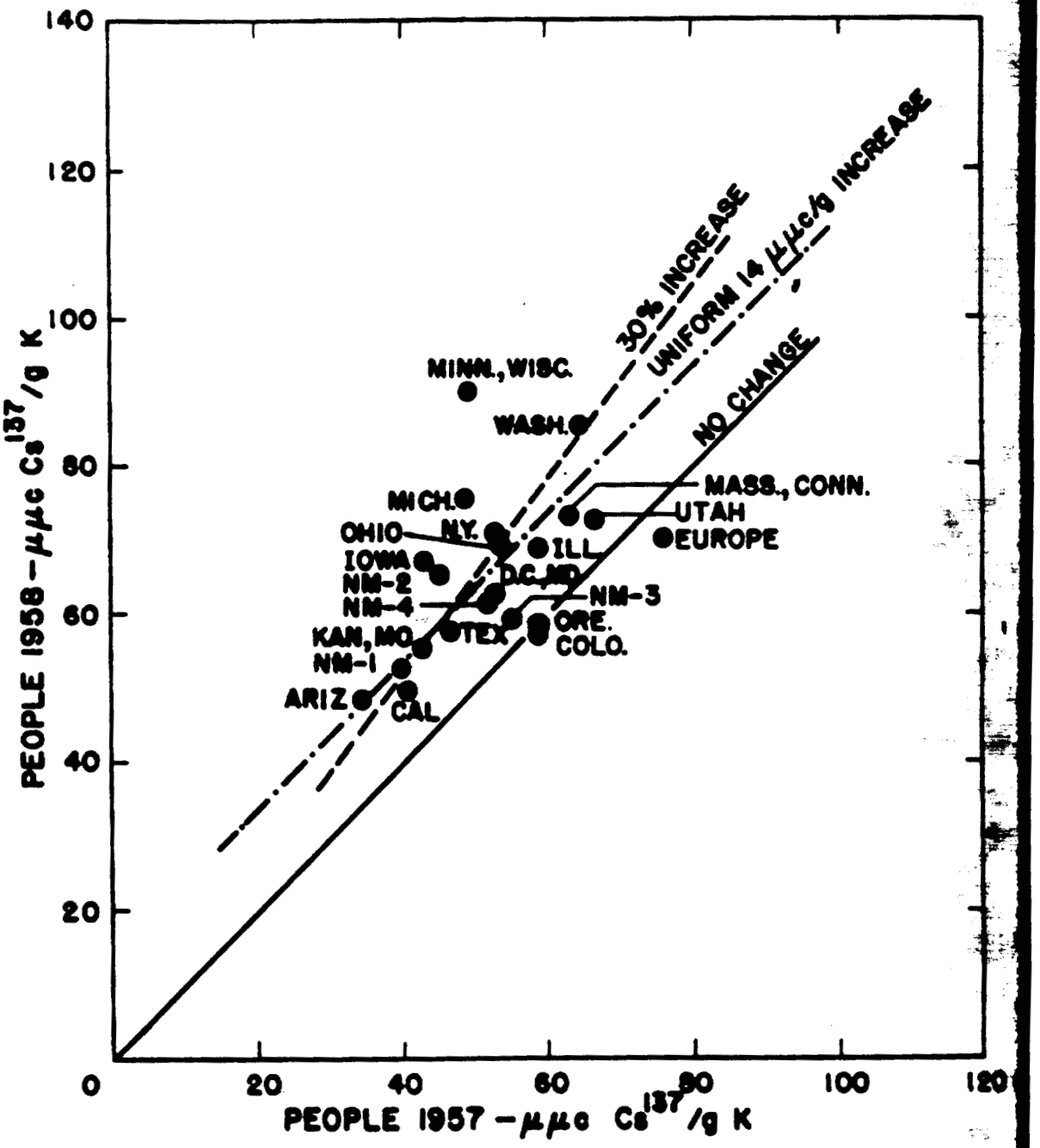

Fig. 5. Cesium^{137} in people (1958 versus 1957).

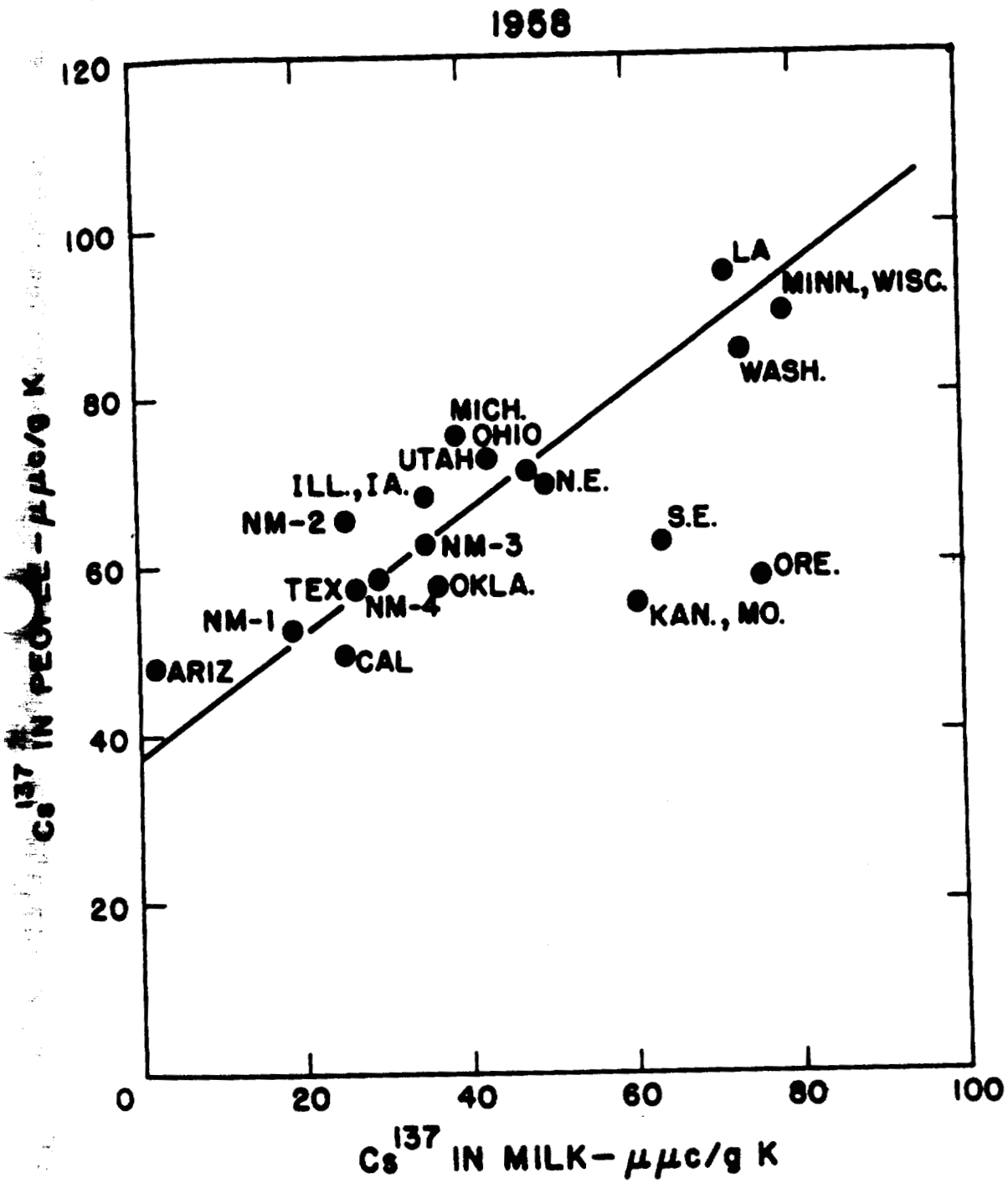

Fig. 6. Cesium^{137} in people versus Cs^{137} in milk (1958).

the Cs^{137} in people appears to be derived from milk.

Since the 1959 results are not yet complete, a detailed analysis is not possible at this time. However, a preliminary summary can be given. The trend of Cs^{137} concentration in milk with time for a few representative locations is shown in Figs. 7 and 8. The amount of structure is surprising for a period during which there was no tropospheric contamination because of the test moratorium. All stations show a sharp peak in milk Cs^{137} activity near midyear. Others show two peaks, one during early spring and another during the early summer. At least three possible factors can contribute to the structure: (a) ecological (e.g., nature of the cows' fodder, silage, graze, etc.); (b) precipitation patterns influencing deposition of fallout; and (c) non-uniform transport from stratosphere to troposphere. An attempt is being made to separate and identify the important factors.

It is interesting that 1959, the first year without weapons tests, has shown both the highest and lowest Cs^{137} concentrations yet observed in milk from several locations. The high peaks in May (e.g., Burlington, Washington, and Franklin, Louisiana) may be a delayed reflection of the intense testing during late 1958, and the stratospheric injection of large amounts of contamination at far norther

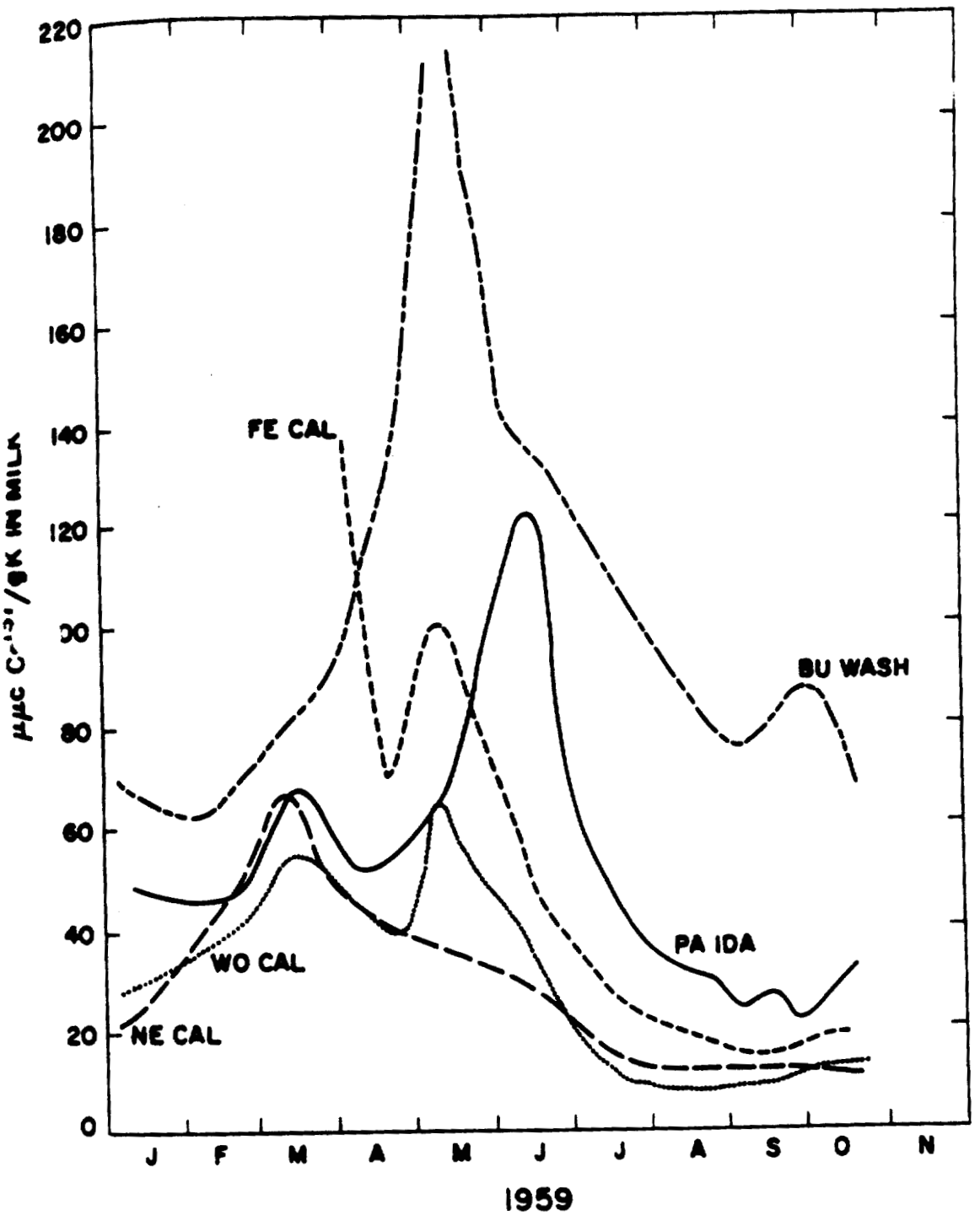

Fig. 7. Cesium^{137} in milk versus date (1959).

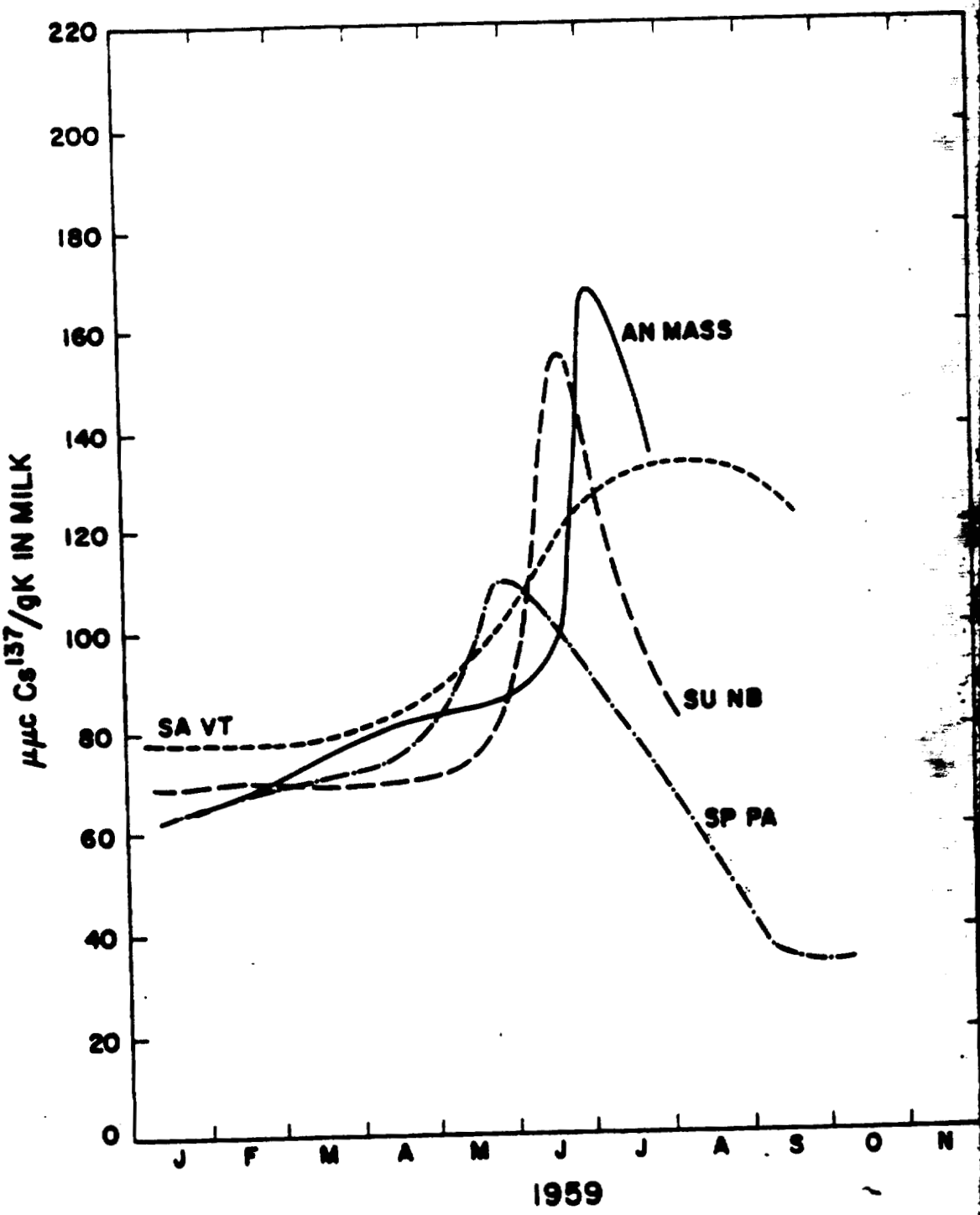

Fig. 8. Cesium^{137} in milk versus date (1959).

latitudes. The unusually low levels being reached by many areas in the fall of 1959 may result from the first "clean" troposphere during the period of observation.

REFERENCES

(1) New York Operations Office, Strontium Program Quarterly Summary Report, Health and Safety Laboratory Report HASL-51 (1958).

(2) New York Operations Office, Strontium Program Quarterly Summary Report, Health and Safety Laboratory Report HASL-55 (1959).

(3) New York Operations Office, Strontium Program Quarterly Summary Report, Health and Safety Laboratory Report HASL-69 (1959).

(4) New York Operations Office, Strontium Program Quarterly Summary Report, Health and Safety Laboratory Report HASL-77 (1960).

(5) E. C. Anderson, B. Clinton, and J. L. Shields, Los Alamos Scientific Laboratory Report LA-2296 (1959).

(6) E. C. Anderson, R. L. Schuch, W. R. Fisher, and W. Langham, Science 125, 1273 (1957).

(7) E. C. Anderson, Science 128, 882 (1958).

Correlation between Cs^{137} Levels in People and Milk from a Specific Area (B. E. Clinton and E. C. Anderson)

Introduction

As mentioned in a previous paper (1), correlation of Cs^{137} levels in people with the levels in milk for the countr in general is made difficult by the sampling problem; people, in general, do not come from the identical area in which the milk is produced and, since sampling consists of measuring visitors from the various areas, frequently the sample size is too small to be representative. For New Mexico, on the other hand, data are available for a sizable local populatio sample since the beginning of 1956, and frequent measurement have been made of the fresh milk production of three major dairies in the Albuquerque area over the same period. The correlation between these data should be considerably better and a more detailed analysis has been made.

Results and Discussion

Because of short-term variations in the milk samples and individual variations in people, the data have been analyzed by quarters and these averages are plotted in Fig. the upper curve giving the results for people, the lower cur that for milk. The number of measurements contributing to the average are indicated in parentheses near each point.

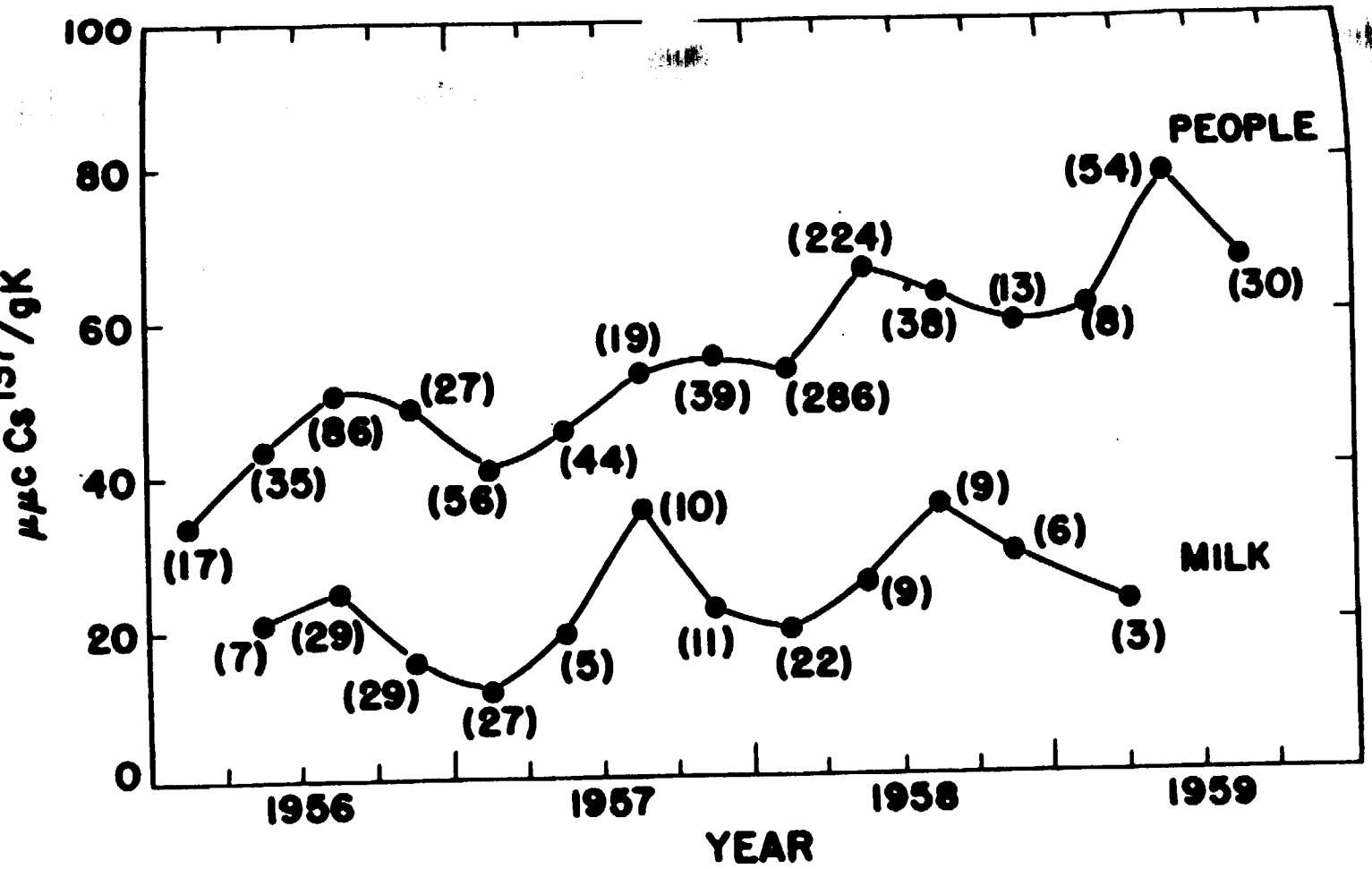

Fig. 1. Quarterly averages of Cs^{137} content in New Mexico people and milk samples.

For both people and milk the level has risen slowly and irregularly over the period of observation, at average rates of 10 and 5 μμc Cs/g K per year, respectively. The pronounced peaks in the milk activity are due, in part to intense tropospheric fallout, as shown by the presence of Ba^{140}/La^{140} activity. In addition, a seasonal pattern in the milk activity is suggested such as might be expected from changes in the food sources of the herd. A somewhat similar, but less intense, structure is evident in the data for people.

A more detailed analysis of the correlation between milk and people is possible from the data shown in Fig. 2, in which the quarterly averages for people are plotted against the milk average for the preceding quarter. Since the biological half-life of Cs^{137} in man is approximately four months, a lag of about one quarter is expected for a product such as milk, which is largely consumed soon after production. The points are numbered and connected in chronological sequence. Of the 11 points, seven show an excellent correlation, while four scatter badly. Careful consideration of these four points is very revealing and suggests a reason for the discrepancy. The two points falling below the curve (points 6 and 10) are associated with milk averages for the third quarters of 1957 and 1958, respectively. These are the points showing the strongest tropospheric peaks in the previous figure (due at

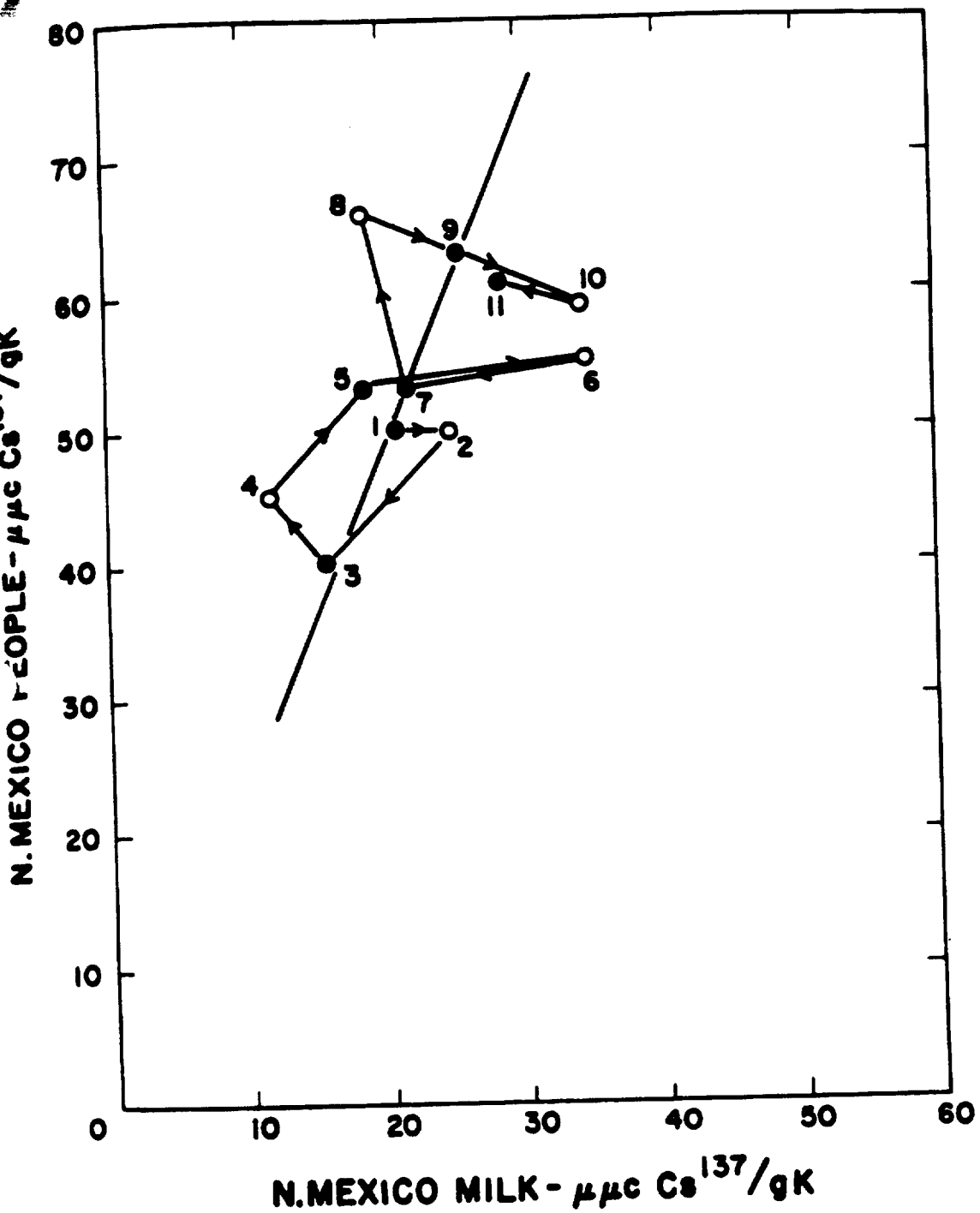

Fig. 2. Cesium^{137} content in New Mexico people versus New Mexico milk averages.

least in part to the United States' Operations Plumbbob and Hardtack). The duration of the milk peak is too short for the activity of the people to rise proportionately, so these periods fall below the curve. The effect is due almost entirely to the milk being too high; the activity of the people remains nearly normal because it has not had time to rise before the milk activity returns to its former lower level. A similar but smaller effect is also to be noted in the third quarter of 1956 (point 2).

The most pronounced failure of people to follow a peak in the milk activity is in the third quarter of 1957 (point 6). In the second quarter of 1958, however, a peak occurs in the activity of people which is not preceded by a milk peak in the first quarter (point 8). In fact, at this time the milk is in one of the seasonal depressions previously mentioned. This aberrant point is due almost entirely to the high human value; the milk is only slightly lower than might be expected. This peak in activity of people may be ascribed to a delayed response to the tropospheric fallout and/or seasonal peak of the previous summer. Foodstuffs such as meat and cereal grain may have a delay of as much as six months between peak contamination and its reflectance in the activity of the consumer. The similarly discordant point for early 1957 (point 4), therefore may be ascribed to the delayed appearance in people of the

1956 summer peak, coupled with the annual winter depression of the milk activity. In this case, the two effects appear to contribute about equally.

If the above explanations are correct, the high milk activity observed in the third quarter of 1958 should be followed by a sharp peak in the human activity during the second quarter of 1959, in spite of the fact that the test moratorium began at the end of 1958. This is indeed the case as is evident from Fig. 1. During this quarter, the New Mexico population average reached an all time high of 79 μμc Cs^{137}/g K. During the third quarter, this average fell to 67, indicating the transitory nature of the increase.

A milk peak was also observed in most of the United States during the summer of 1959 (see following section on Correlation of Cs^{137} and Sr^{90} Levels in Milk). While no Ba^{140}/La^{140} activity accompanied this peak, it is not certain that the increase is due entirely to seasonal effects. This uncertainty is due to the unusual nature of the weapons tests in the fall of 1958; that is, the injection of large amounts of debris into the Arctic stratosphere in which an unusually short residence time of perhaps 6 months is expected. Data taken during the spring and summer of 1960 should help clarify this point.

The above explanations are admittedly ad hoc and cannot

be regarded as definitively proven by the data. However, they do present a consistent picture and have the advantage of suggesting further experimentation. If true, they also illustrate the complexity of the situation and the necessity for complete studies on statistically significant numbers of people and on the major components of their diet, in order to be certain of the factors operating.

REFERENCE

(1) E. C. Anderson, Science 128, 882 (1958).

Correlation of Cs^{137} and Sr^{90} Levels in Milk (E. C. Anderson with A. R. Schulert, Lamont Geological Observatory)

Introduction

Monitoring the Sr^{90} levels in the country's milk supply is difficult, time-consuming, and expensive. Since no gamma rays are emitted by Sr^{90}, a chemical separation must be performed preparatory to low level beta counting. $Cesium^{137}$, on the other hand, can be easily and rapidly determined without processing by gamma ray spectrometry with either a liquid scintillator or a sodium iodide crystal counter. It is very attractive, therefore, to consider the possibility of estimating Sr^{90} concentration from the Cs^{137} analysis.

Because of the great chemical differences between strontium and cesium, it was clear a priori that a correlation between their levels in milk, if any, would not be rigorous and would be subject to a number of variations (including soil type and composition, nature of forage eaten by the dairy herds, etc.). High precision, however, may not be required for some monitoring problems, and frequently the point of principal interest is the average concentration of the radioactive nuclide over say a 12-month period rather than the concentration in individual samples. Sharp fluctuations in Sr^{90} levels are less important than the general trend in the activity level. For this reason, it seemed worthwhile to make a fairly detailed

study of the Sr^{90} and Cs^{137} concentrations in milk samples from various parts of the United States.

Results and Discussion

Weekly aliquots were taken from the LASL file of dry milk samples beginning in early 1957, composited by months, and sent to the Lamont Geological Observatory for Sr^{90} and calcium analyses. Results of this program have been reported in the Lamont Geological Observatory's annual report (1). They concluded that "the Sr^{90} is varying essentially independently of the Cs^{137} in time so that measurement of the latter cannot be used to obtain quantitative estimation of the Sr^{90}."

Although a rigorous quantitative correlation in individual samples does not seem to exist, crude relationships do seem to appear between averages for a given area or region. A general area correlation is indicated by comparison of our 1958 Cs^{137} values for Canadian milk with the Sr^{90} values from the same area reported by Grummitt, et al (2). Table I shows the relative rating of Sr^{90} and Cs^{137} average levels in four major Canadian milk producing areas.

Figure 1 presents the LGO-LASL data plotted as Sr^{90} versus Cs^{137}, rather than as strontium to cesium versus date. While the scatter is indeed considerable, the correlation between the two variables is rather good as biological

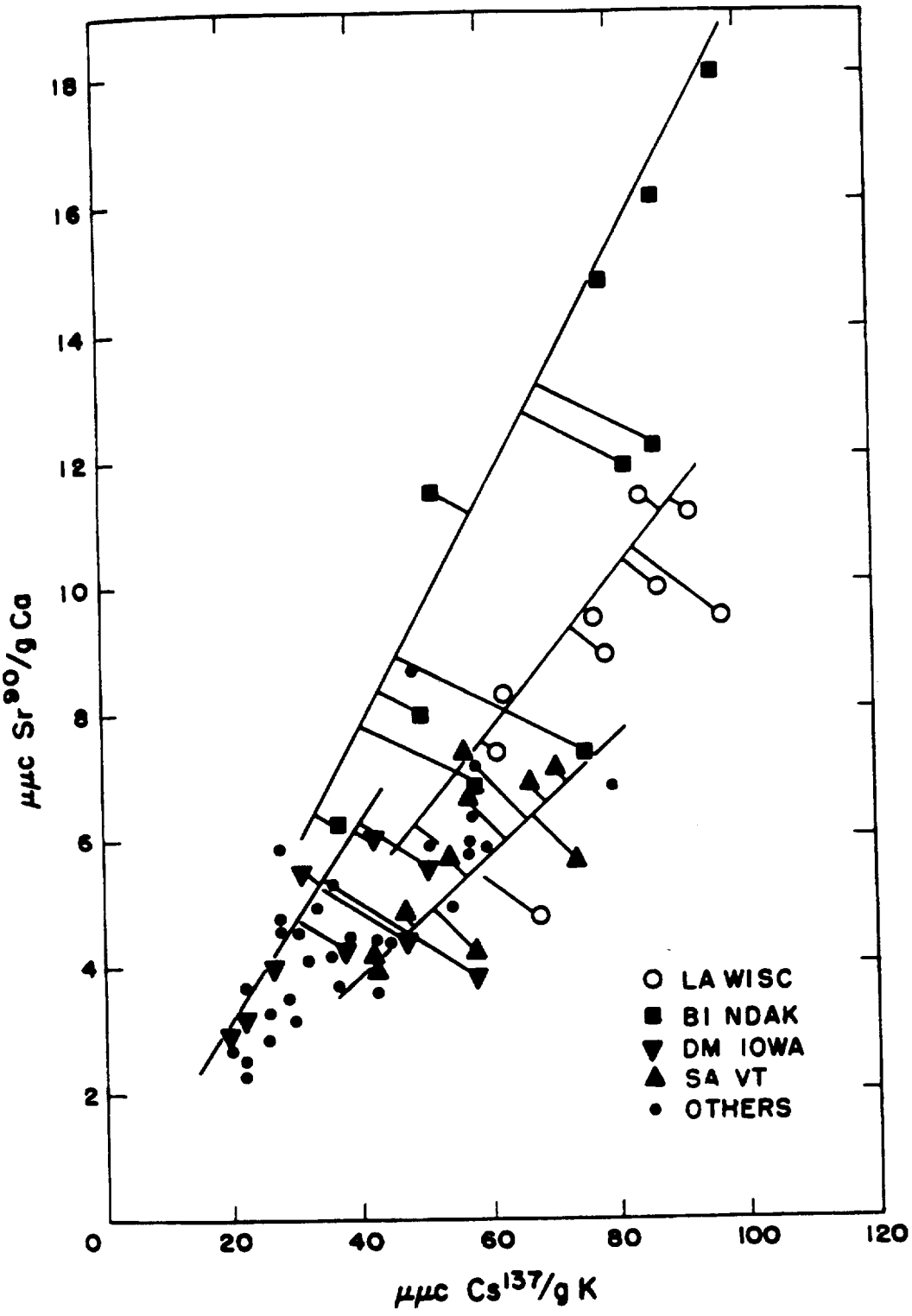

Fig. 1. Strontium^{90} versus Cs^{137} in milk.

TABLE 1. RELATIVE Sr^{90} AND Cs^{137} AVERAGE LEVELS IN FOUR MAJOR CANADIAN MILK PRODUCING AREAS (1958)

Area	Relative Average Level	
	Sr^{90} (2)	Cs^{137}
Ontario	0.6	0.69
Western Canada	1.0	0.87
Maritimes	1.2	1.17
Eastern Quebec	1.5	1.89

experiments go. The explanation of the apparent contradiction of the two independent interpretations appears to be a difference in the precision standards used. Referring again to the LGO report, specifically to their Fig. IV-A for Bismarck, North Dakota, one can note that over the period from March 19 through August 1958, the Sr^{90} concentration in the milk rose from 6 to 22 μμc/g Ca (a factor of 3.7), while over the same period the Cs^{137} changed from 37 to 80 μμc/g K (a factor of 2.2). The ratio changed on the average from about 0.15 to 0.23 (a factor of 1.5). The range of variation of the ratio (from 0.10 to 0.28) supports the Lamont conclusion that measurements of Cs^{137} cannot be used to obtain quantitative estimation of the Sr^{90}. It also appears to be true that a certain portion of the Sr^{90} variation is not paralleled by a corresponding variation in the Cs^{137}. However, it is our contention th

the basic long-term increase over this period (as well as the subsequent decline during 1959) is common both to Sr^{90} and Cs^{137}, and that within an admittedly large uncertainty it is possible to estimate one from the other. Whether the correlation is sufficiently good to be of practical value will depend on the problem at hand. Where accurate Sr^{90} values are required, as for example in the estimation of discrimination factors, calculations based on Cs^{137} levels are clearly useless. For routine monitoring, for example as a means of interpolation between direct Sr^{90} determinations at yearly intervals or in areas not otherwise sampled, the method may be of some utility.

As Fig. 1 clearly demonstrates, the correlation coefficient is not a constant for all geographic locations but varies significantly in different parts of the country, the range between Vermont and North Dakota being a factor of 2. Figure 2 shows the geographical variation in the observed average Sr^{90}/Cs^{137} ratio in milk; a calcium to potassium ratio of 0.69 is assumed.

It is possible to estimate crudely the expected value of the Sr^{90}/Cs^{137} ratio. If both strontium and cesium in milk are derived entirely from soil uptake, then the ratio should be given in accordance with the following expression:

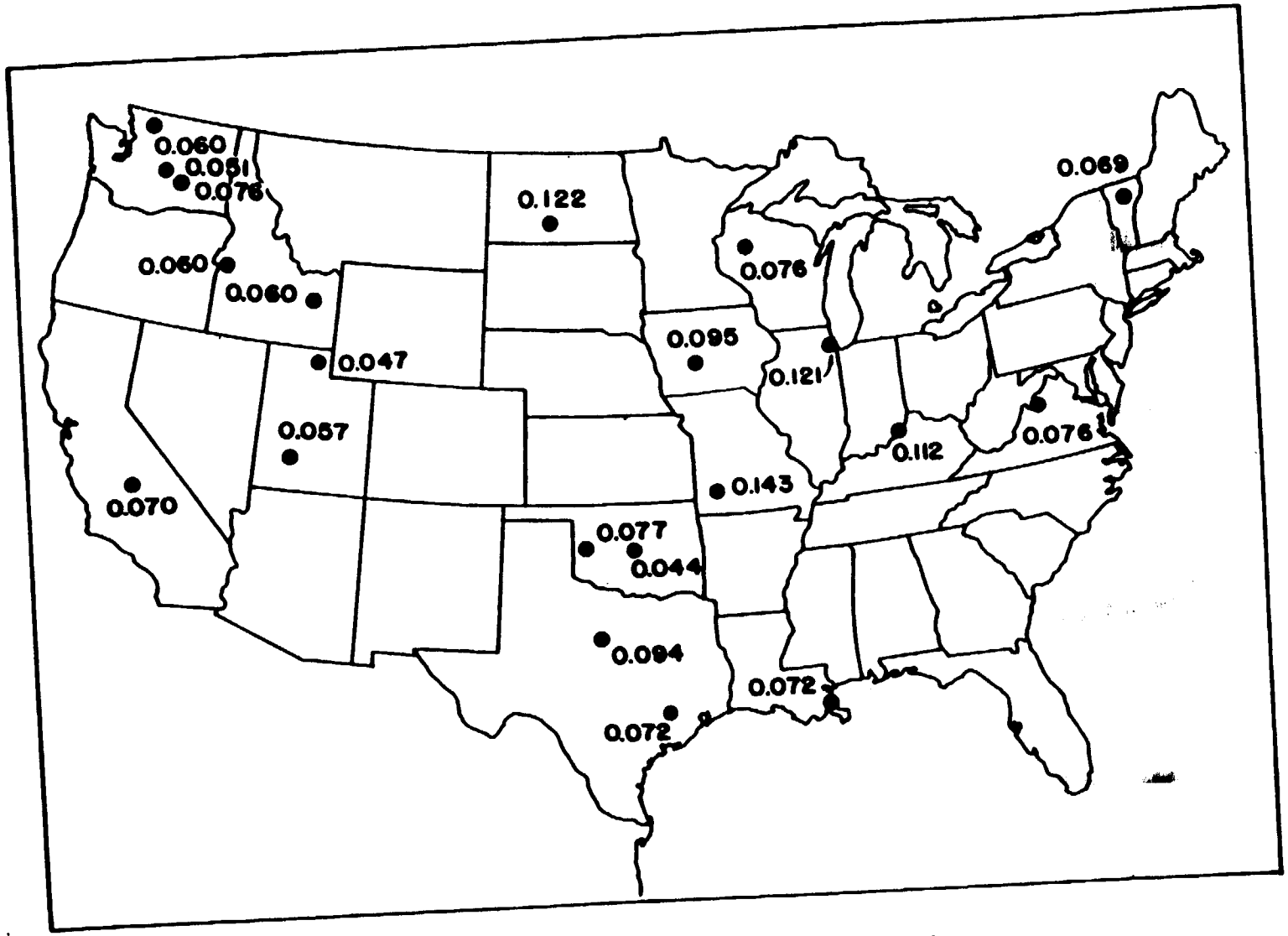

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Fig. 2. Geographical variation in Sr^{90}/Cs^{137} ratios in United States milk.

$$\left[\frac{Sr^{90}}{Cs^{137}}\right]_{milk} = \left[\frac{Sr^{90}}{Cs^{137}}\right]_{fallout} \times \left[\frac{K}{Ca}\right]_{soil} \times DF_1 \times DF_2 \times \left[\frac{Ca}{K}\right]_{milk} \quad (1)$$

where DF_1 is the ratio of the discrimination factors of strontium and cesium relative to calcium and potassium in going from soil to plants, and DF_2 is the corresponding ratio between plants and milk (3). The arithmetic solution is:

$$\left[\frac{Sr^{90}}{Cs^{137}}\right]_{milk} = 0.55 \times 1/20 \times 0.7/0.01 \times 0.13/2 \times 0.100/0.145$$
$$= 0.086$$

All these factors are more or less uncertain, the principal sources of error probably being in the $\left[\frac{K}{Ca}\right]_{soil}$ and DF_1 values (3).

An alternative simple model assumes that both Sr^{90} and Cs^{137} are derived only from direct fallout (by foliate absorption by plants and/or direct ingestion by the cow). The ratio is then given by Eq. 2:

$$\left[\frac{Sr^{90}}{Cs^{137}}\right]_{milk} = \left[\frac{Sr^{90}}{Cs^{137}}\right]_{fallout} \times \left[\frac{K}{Ca}\right]_{plant} \times DF_2 \times \left[\frac{Ca}{K}\right]_{milk} \quad (2)$$

Here, the principal numerical uncertainty lies in the choice of the potassium to calcium ratio for plants. For shallow-rooted grasses, this value is about 4.7 whereas for more

deeply rooted hay crops (such as alfalfa and clover) it is about 1.4. The calculated ratio of Sr^{90}/Cs^{137} in milk is, therefore, 0.116 to 0.035 (depending on the potassium to calcium ratio chosen for the plants). Note that a possible seasonal variation in the ratio may result from the grass-hay difference as the feeding regimen of the dairy herds undergoes seasonal change.

For the more probable case in which both soil uptake and direct fallout contribute, a general calculation is not possible. However, the agreement of the range of calculated values with the observed range of 0.04 to 0.14 is encouraging, and this suggests that a detailed calculation based on actual potassium to calcium ratios in soils and plants of a given milk-shed or farm would be profitable.

REFERENCES

(1) A. R. Schulert, Lamont Geological Observatory, Columbia University, Palisades, New York. Annual Report (September 1959).

(2) W. E. Grummitt, A. P. James, and H. B. Newcombe, Atomic Energy of Canada Limited Report CRC-850 (1959).

(3) W. H. Langham and E. C. Anderson, Health Physics 2, 30 (1959).

Total Body Potassium in Man (B. E. Clinton, W. H. Langham, and E. C. Anderson)

Introduction

Measurements on the average potassium concentration of the human body (determined by whole body counting of the K^{40} isotope) as a function of age for 1590 subjects have been published (1). Interest in body potassium has continued because of its possible relationships to lean, protoplasmic mass of the body, to muscular development, and to physiology of aging. Whole body counting of the gamma rays from the naturally occurring isotope K^{40} permits determination of the body potassium content in only 300 seconds. If indeed the body's potassium is confined to the lean, oxidizing, protoplasmic mass, it should correlate with basal metabolic rate and should give information regarding a fundamental body parameter and its variation in health and disease. Potassium measurements may be used also indirectly to estimate body fat.

Results and Discussion

During the past few months attempts have been made to check LASL whole body potassium measurements with those made elsewhere by the same and other methods.

A comparison of LASL results on 21 male subjects (aged 19 to 20 years) with similar results (using a NaI crystal counter) reported by McNeill and Green (2) for thirty 19-year old subjects showed excellent agreement. They reported an average potassium content of 2.12 ± 0.02 g/kg, compared to our average of 2.15 ± 0.05 g/kg. In addition, 1 subject [redacted] of the United Kingdom Atomic Energy Research Establishment at Harwell has kindly sent the results on himself as determined at four installations (including LASL). These comparative results are shown in Table 1.

TABLE 1. TOTAL BODY POTASSIUM OF [redacted] AS INDEPENDENTLY MEASURED AT FOUR DIFFERENT INSTALLATIONS

Installation and Method	Total Potassium (g)
LASL (liquid scintillator)	152
Leeds (plastic scintillator)	153
Harwell (NaI crystal)	149
ANL (NaI crystal)	146

Five subjects whose total body potassium had been determined at the Naval Radiological Defense Laboratory using K^{42} isotope dilution were measured in the Los Alamos counter, and a comparison of the two sets of results are shown in Tabl

TABLE 2. COMPARISON OF TOTAL BODY POTASSIUM MEASURED BY K^{40} COUNTING (LASL) AND BY K^{42} ISOTOPIC DILUTION (NRDL)

Subject	LASL		NRDL*	
	Wt (kg)	K (g)	Wt (kg)	K (g)
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

*We are grateful to [redacted] and [redacted] for the opportunity to make this comparison.

In only one case is the disagreement between the two methods greater than 5 per cent. Note also that in some subjects there were rather large weight changes between the two sets of measurements. Since the last four persons were athletes (weight lifters), some differences in total potassium may be expected on the basis of intensity of training and degree of physical fitness at the times of measurement. One individual [redacted] had been training for about 30 days in preparation for Olympic wrestling competition.

REFERENCES

(1) E. C. Anderson and W. H. Langham, Science 130, 713 (1959).

(2) K. G. McNeill and R. M. Green, Can. J. Phys. 37, 683 (1959).

Calibration of Large Volume Detectors for Absolute Measurement of Cs^{137} (E. C. Anderson and M. A. Van Dilla)

Introduction

The problem of absolute calibration of large volume detectors for Cs^{137} measurement is difficult because of the complex decay scheme and differing methods of absolute standardization. Two types of standards have been investigated: those based on gamma ray measurements and those based on absolute beta counting. For calculation of true microcuries of Cs^{137}, it is assumed that the gamma ray abundance in the decay scheme is 85 per cent (95 per cent beta decay to Ba^{137m}, 10 per cent internal conversion of the 0.662-Mev gamma ray) and that total electron emission per disintegration is 1.095 (the conversion electrons of Ba^{137m} are emitted with a half-life of 2 minutes and are, therefore, counted by the absolute beta method).

Methods and Results

Table 1 summarizes recent recalibrations made with new sets of independent Cs^{137} standards. Three of the standards used for these recalibration studies were prepared at the Walter Reed Army Institute of Research (designated WRAIR 3, 4, and 7) from aliquots of a National Bureau of Standards'

TABLE 1. CESIUM^{137} CALIBRATIONS OF THE FIRST MODEL LOS ALAMOS LARGE VOLUME LIQUID SCINTILLATION DETECTOR (HUMCO I)

Standard	Nominal Activity (d/sec)	Corrected for Decay (d/sec)	Humco I (c/sec)	Efficiency	Corrected Efficiency*
WRAIR 3	47,400	45,700	6,527	0.143	0.150
WRAIR 4	3,790	3,540	612	0.167	0.167
WRAIR 7	47,400	45,700	6,659	0.145	0.153
				Average	0.157
LASL 1	24,200	23,200	3,690	0.159	0.159
LASL 1a	2,960	2,850	448	0.158	0.158
LASL 3	23,700	22,800	3,668	0.161	0.161
LASL 4a	2,950	2,830	436	0.155	0.155
LASL 1 and 3	47,900	46,000	6,914	0.150	0.158
				Average	0.158
Humco 1	4,260	3,920	746	0.190	0.190

*Corrected for coincidence loss.

Cs^{137} solution, and four standards (LASL 1-4) based on absolute beta counting were prepared by Group J-11 of the Los Alamos Scientific Laboratory. The LASL standards were cross-checked against a Nuclear-Chicago Cs^{137} gamma standard, and the two methods showed agreement to $\pm$ 3 per cent. The Humco I standard has been the working standard used for all human counter measurements thus far published. It was calibrated in January 1956 by absolute beta counting. It appears from this table that the absolute calibration on this standard is in error by 21 per cent, and the true source strength is

4260 x 0.190/0.158, or 5140 d/sec. The magnitude of this discrepancy has been verified by measurement of the Humco 1 standard against LASL 1 on the crystal spectrometer. An activity ratio of 0.191 was obtained with the spectrometer, compared with a ratio of 0.202 measured with the liquid scintillator.

The actual correction to be applied is smaller than this, since in the initial interpretation of the beta standardization it was not realized that the conversion electrons were being counted. Since February 1, 1956, the IBM program for calculating Cs^{137} counting efficiency has used 3900 gamma rays per second as the strength of the Humco 1 standard and 85 per cent as the gamma ray abundance, which is equivalent to a source strength of 4590 d/sec. The present recalibration indicates that all results should be raised by 5140/4590 or 1.12. This correction applies to all published results except the 1957 data summary as reported in Science (1). These data were based on an earlier computer program and should be multiplied by 1.18 to put them on the same basis as the other data, or by 1.18 x 1.12 = 1.32 to put them on the new recalibrated basis.

A re-analysis of calibration data obtained with aqueous phantoms, sugar phantoms, and humans ingesting tracer doses of Cs^{137} has revealed the need for an additional correction

factor in the computer program. It was early established that the Cs^{137} counting efficiency did not depend markedly upon the weight of the subject or sample, the variation between 40 and 170 pounds being within the range $\pm$ 7 per cent. Since this effect was small compared with other sources of error and with the random variations of the subjects being measured (standard deviation of the population distribution curve = 36 per cent); the program was written with a Cs^{137} efficiency independent of sample weight. Calibration is based on the daily measurement of the Cs^{137} standard placed in the center of an 88-pound sugar phantom. Self-absorption for a central source is higher than for a distributed source by 22 per cent (in the case of people) and 38 per cent (in the case of dry milk samples). A reduction in the calculated absolute activity by these factors is, therefore, indicated.

Combining the self-absorption correction with the recalibration of the standard gives a total correction factor of 0.915 for people and 0.810 for milk samples. (For the 1957 summary in Science, the total correction factors are 1.08 and 0.96, respectively.) It should be noted that these corrections refer to the absolute value of the Cs^{137} concentration and that all values are changed by the same factor. The relative precision is not affected by the change,

and correlations with rainfall, geographic locations, etc., are unchanged. The discrimination factor between milk and man, previously reported as 1.8, however, is increased to 2.0 because of the 13 per cent difference in the corrections for these samples.

It is planned to reinvestigate the entire Ca^{137} calibration problem as soon as Humco II is operational (see below). With the improved precision expected from the new counter, it is desirable to refine the absolute calibration proportionally. Pending the results of this detailed study, the present computational program will not be changed to include any of the above effects.

REFERENCE

(1) E. C. Anderson, Science 128, 882 (1958).

Zinc^{65} in Cyclotron Workers (M. A. Van Dilla and M. J. Engelke, H-1)

Measurement of LASL cyclotron workers in November 1958 in Humco I (1) revealed the presence of radioactivity in excess of normal K^{40} in the 1- to 2-Mev region. This excess was identified as Zn^{65} using the low-level gamma ray spectrometer (2,3). Figure 1 is a typical spectrum showing the characteristic Zn^{65} peak at 1.11 Mev in addition to the usual Cs^{137} and K^{40}.

Zinc^{65} can be produced in large quantities in cyclotrons accelerating deuterons by the reaction $Cu^{65}(d,2n)Zn^{65}$ on copper dees and other parts. In fact, this reaction has been used for producing high specific activity Zn^{65} (4) for biological tracer work. In addition, deuteron reactions can produce 291-day Mn^{54}, and He^3 reactions can produce 53-day Be^7.

A repair operation in which the machine is opened and the dees removed is referred to as a "rollback" at this Laboratory. When this occurs, a major clean-up usually follows. During rollbacks, gamma ray spectra of filter papers* from the air samplers have shown Zn^{65}, but in addition peaks very close to the energy of Be^7 (0.48 Mev) and Mn^{54} (0.84 Mev). The surface of a nearby workbench top showed the same radioactivities, as

*HV-70 filter paper was used; manufactured by Hollingsworth and Vose Co., East Walpole, Mass.

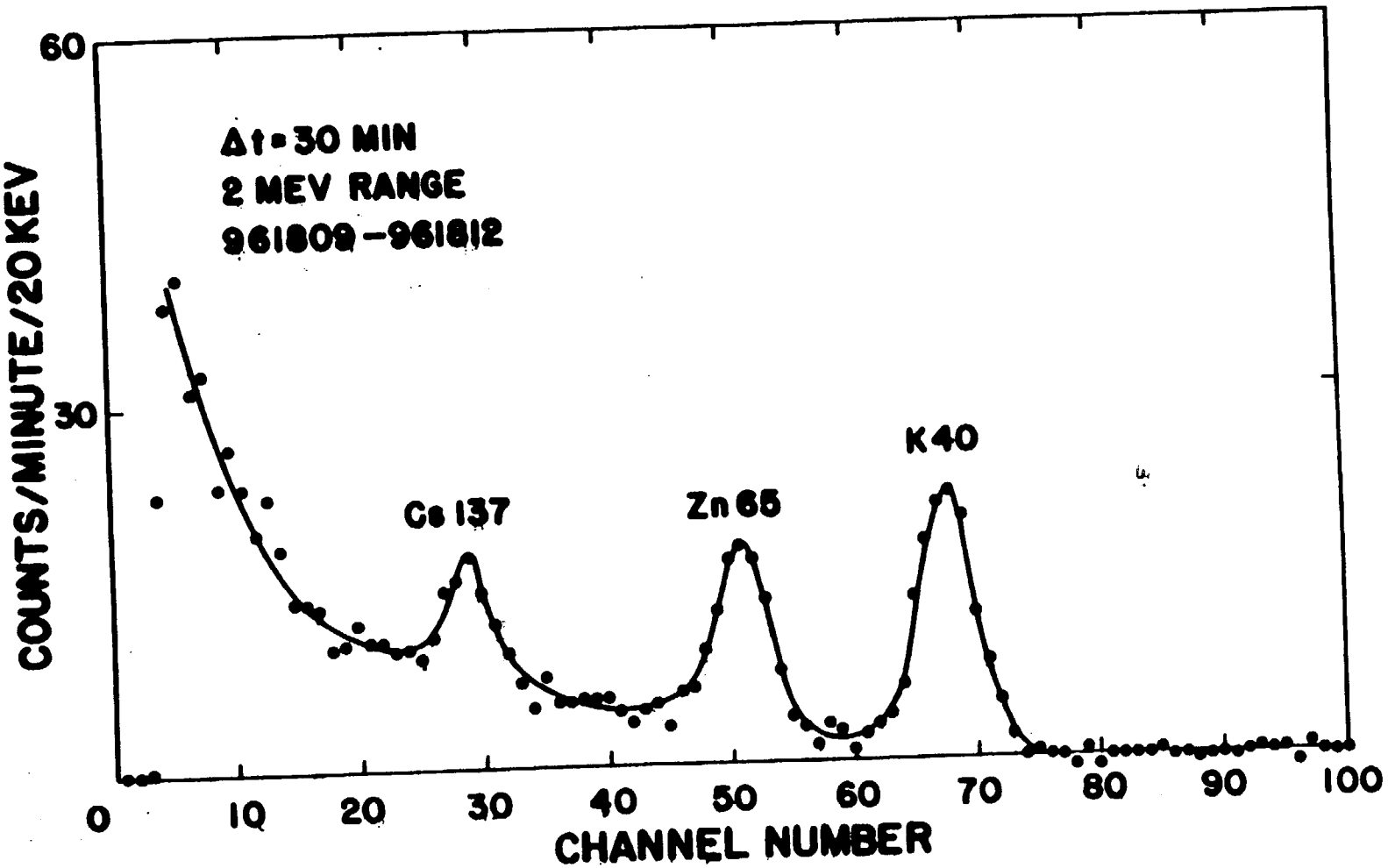


Fig. 1. Gamma ray spectrum of cyclotron worker [redacted]

did the cyclotron deflector and ion source holder. Assuming that the 0.48-Mev peak is actually Be^{7}, then this and Zn^{65} were the predominant radionuclides observed and their abundances were comparable. Tools (screwdriver, etc.) were similarly contaminated; the amount of Zn^{65} was of the order of 0.1 μc.

Gamma ray spectra of several of the cyclotron personnel most involved in rollbacks have been measured serially since the exposure was discovered in November 1958. These data are shown in Fig. 2. The maximum body burdens observed were about 0.1 μc, which is quite trivial compared with the maximum permissible body burden of 60 μc (5). The effective biological half-time is slightly less than the half-time of physical decay ($T_{1/2}$ = 245 days). Recontamination, however, may have occurred as a result of rollbacks during the period of study, and results on one individual seem to show this. Doses of Zn^{65}, administered orally to people (6), were efficiently absorbed, and retention ranged from 50 to 80 per cent several months after ingestion. The effective biological half-time was 158 days. Urinary excretion several weeks after ingestion was only about 0.05 to 0.1 per cent of the body burden. Analysis of urine samples of the five cyclotron workers listed in Fig. 2 showed that if any Zn^{65} were present, the amount was less than 0.2 to 0.3 per cent of the body

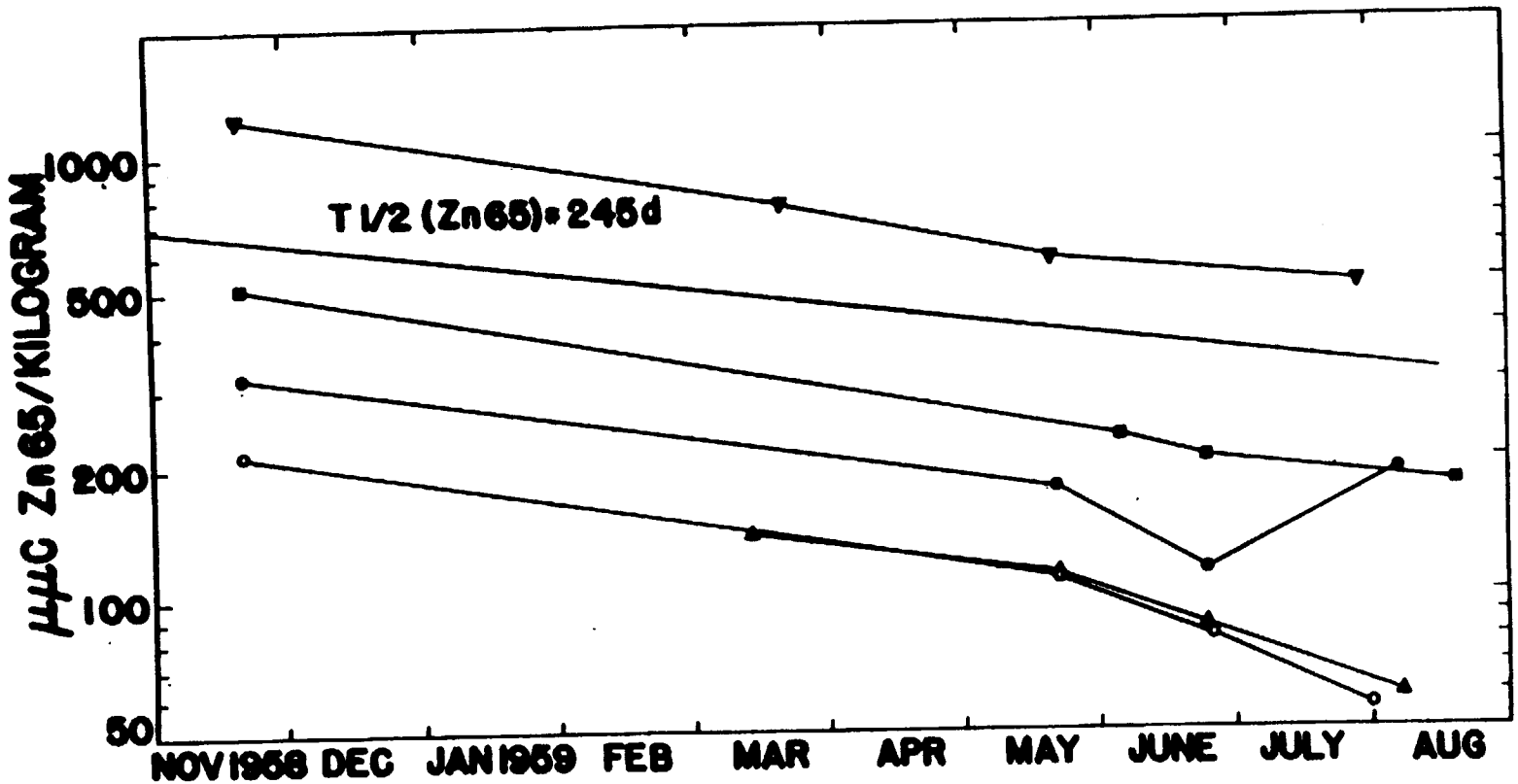


Fig. 2. Elimination curve of Zn^{65} in cyclotron workers.

burden in a 24-hour sample. It is interesting that only once did the cyclotron personnel show any radioactivity other than Zn^{65} and the usual Cs^{137} and K^{40}. This was at the end of June 1959, when they showed a peak at 0.46 Mev, which is very close to the Be^{7} energy. This may have been skin or hair contamination subsequently washed off.

To minimize recontamination during rollbacks, when particulate matter may become air-borne, protection is afforded by use of shoe coverings, gloves, coveralls, caps, and respirators. The routes of entry into the body are not certain, but probably one of them is inhalation. All the personnel were fitted for respirators (the half-mask type being preferred by the group). External exposure has been kept within permissible levels by using shielding, distance, and limited working times.

It is probable that many cyclotron workers and perhaps those working on other accelerators (or reactors) have small Zn^{65} burdens. It has been reported in two cyclotron workers at the Massachusetts Institute of Technology (7). A survey of those potentially exposed at other installations would be of interest from the viewpoint of good health physics practice and better understanding of the metabolism of radionuclides in the human body.

REFERENCES

(1) E. C. Anderson, I.R.E. Trans. Nucl. Sci. NS-3(4), 96 (1956).

(2) E. C. Anderson and M. A. Van Dilla, I.R.E. Trans. Nucl. Sci. NS-5(3), 194 (1958).

(3) M. A. Van Dilla, Nucleonics 17(11), 150 (1959).

(4) C. J. Maletskos and J. W. Irvine, J. Chem. Phys. 19, 796 (1951).

(5) National Bureau of Standards Handbook No. 69, Maximum Permissible Body Burdens and Maximum Permissible Concentrations of Radionuclides in Air and in Water for Occupational Exposure (1959).

(6) C. R. Richmond, W. H. Langham, and M. A. Van Dilla, unpublished data (1959).

(7) Massachusetts Institute of Technology, Annual Progress Report, Contract AT(30-1)-952 (1959).

Zinc^{65} and Zirconium^{95} in Food (M. A. Van Dilla)

Zinc^{65} can be produced by bomb tests and nuclear reactors by neutron interaction with stable zinc and, in special cases, its presence in living organisms has been observed (1-3). We have now detected this isotope in muscle and liver samples from cattle raised in Nevada (4), and also in commercial hamburger (lean ground beef shoulder) and beef liver from the southwestern area (Table 1). Zirconium^{95} and niobium^{95}, a fission product pair very common in fallout, were also detected in the Nevada and locally procured liver samples, but not in the muscle or hamburger. Examination of milk samples from two areas of relatively high fallout (northwest Washington and Louisiana in May 1959) showed little or no Zn^{65}. Careful measurement of two people at this Laboratory failed to reveal anything but the usual Cs^{137} and K^{40}.

TABLE 1. APPROXIMATE RADIOACTIVITY OF COMMERCIAL BEEF LIVER AND HAMBURGER OBTAINED LOCALLY (75-pound samples)

Sample	μμc/kg			Potassium
	Zinc^{65}	Zirconium^{95}	Cesium^{137}	(gm/kg)
Beef Liver	50	30	180	2.8
Hamburger (lean ground beef shoulder)	30	< 6	200	2.3

The fact that small amounts of Zn^{65} appear in cattle should not be surprising. It is known that this radionuclide is absorbed with high efficiency from the gastrointestinal tract of cows (5); total urinary and fecal excretion in the first four days was about 25 per cent. Measurements at this Laboratory (6) of Zn^{65} retention in people after oral ingestion showed that most of the isotope is absorbed, and retention ranged from 50 to 80 per cent (after correction for physical decay) several months after ingestion. In the case of the very heavy fallout in the Marshall Islands following the thermonuclear detonation of March 1954, Zn^{65} and Cs^{137} were found (1) in the Rongelap residents in roughly equal amounts (ranging up to 0.5 μc). Thus, large quantities of Zn^{65} can be produced in bomb tests, and its entry into cattle and people is to be expected. It will not be a surprise if more extensive measurements now or in the near future reveal small amounts in people.

The main point about the milk results is that the Zn^{65} content is about an order of magnitude or more below meat. It should be noted that the milk samples were obtained from different places than the meat, which comes from the southwestern area (mainly Texas, Oklahoma, New Mexico, and Colorado). In addition, Zn^{65} production may not be well correlated with formation of fission products, since the former is the result

of neutron interaction with stable zinc in bomb parts and/or the surroundings. Thus, it is possible that the milk received its radioactivity from fallout with a low Zn^{65} ratio, while the meat represents fallout with a high ratio.

Grazing animals ingest large amounts of radioactive cerium, ruthenium, and zirconium as foliar contamination. Gastrointestinal absorption is appreciable only for Zr^{95} and Nb^{95}, and retention is high (7) at least in rats. The liver is a major organ of deposition. Expected levels in milk and people would be much reduced because of the large discrimination factors that exist at each step up the food chain.

REFERENCES

(1) J. E. Rose and L. D. Marinelli (eds), Argonne National Laboratory Report ANL-5755, pp. 53-60 (1957).

(2) R. W. Perkins and J. M. Nielsen, Science 129, 94 (1959).

(3) M. A. Van Dilla and M. J. Engelke, Science (elsewhere in this report).

(4) M. A. Van Dilla and G. R. Farmer, Radioactivity of Nevada Cattle (elsewhere in this report).

(5) H. Bergh, Det Kongelige Norske Videnskabers Selskab Forhandlinger 23(13), 49 (1950).

(6) C. R. Richmond, W. H. Langham, and M. A. Van Dilla, unpublished data (1959).

(7) C. R. Richmond, D. W. Martin, and M. A. Van Dilla, unpublished data (1959).

Measurements on Irradiated Meat (M. A. Van Dilla and E. C. Anderson, with R. A. Glass and H. Smith, Stanford Research Institute)

Samples of beef irradiated with 12 megarads of 24-Mev electrons on August 7, 1959, were measured with both Humco I and the sodium iodide gamma ray spectrometer at LASL. The gamma ray spectrum of a typical sample is shown in Fig. 1. The principal activity induced by the irradiation was Na^{22}, produced at a concentration of about 2×10^{-14} curies per gram per megarad. The samples were also measured by H. May at the Argonne National Laboratory and provide a comparison between the methods (Table 1).

TABLE 1. RADIOACTIVITY OF MEAT SAMPLES IRRADIATED WITH 12 MEGARADS OF 12-MEV ELECTRONICS

	ANL Crystal	LASL Crystal	LASL Liquid Scintillator
Unirradiated Sample			
Grams K/kg	3.35	2.54	2.31
$\mu\mu c$ Cs^{137}/g K	96	123	103
Irradiated Samples			
$\mu\mu c$ Na^{22}/g meat	0.29	0.26	----
	0.21	0.17	----

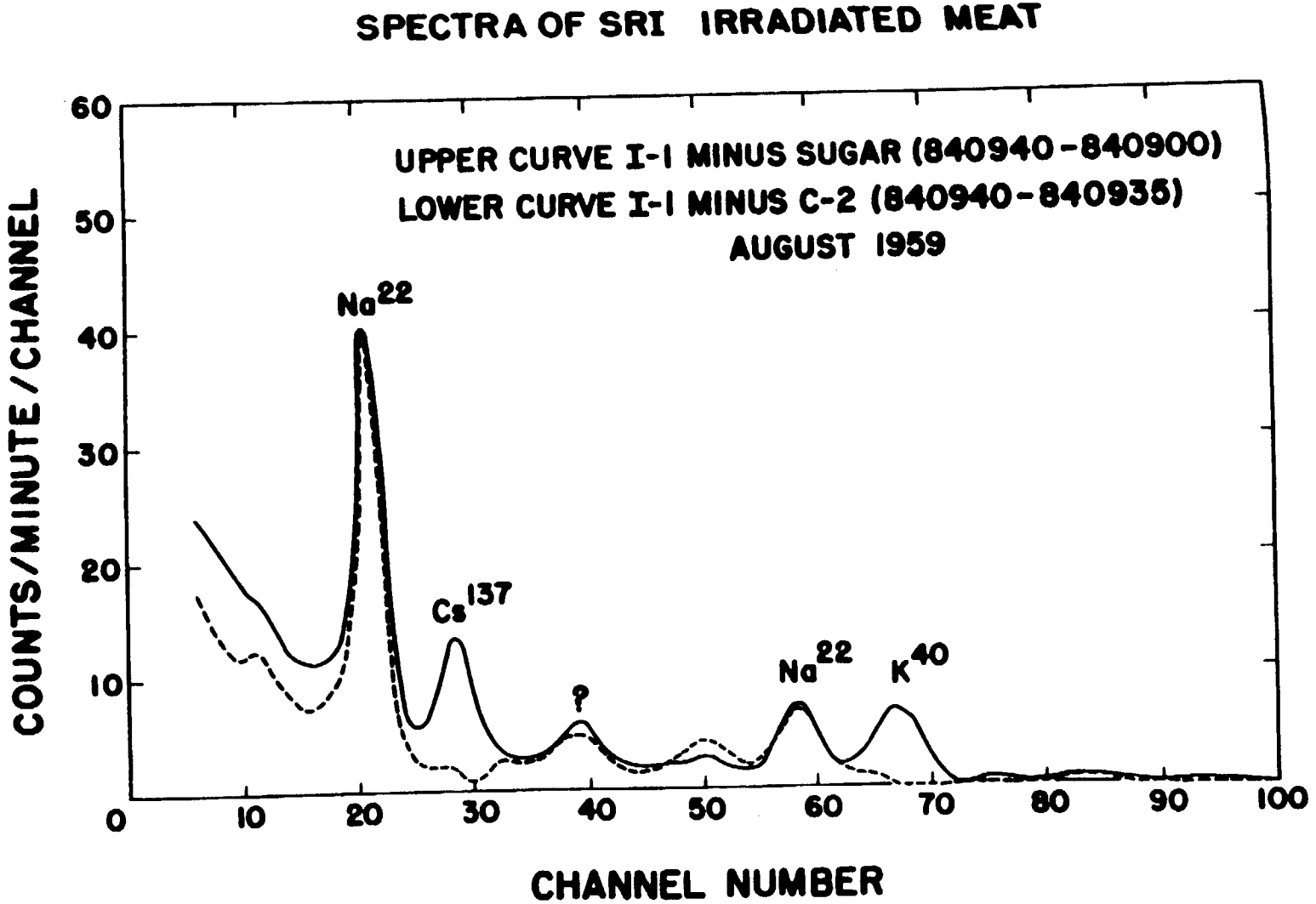


Fig. 1. Gamma ray spectrum of irradiated meat.

Radioactivity of Nevada Cattle (M. A. Van Dilla and Major G. Farmer, on assignment to the U.S.A.E.C.)

In July 1957, a project was started by the U. S. Atomic Energy Commission to find out whether cattle raised in the vicinity of the Nevada Test Site showed any radiation effects, or were ingesting appreciable amounts of fission products and depositing them in their tissues. At present, herds are maintained at Delamar Valley, Caliente, Nevada, and at Knoll Creek, Contact, Nevada, by the University of Nevada, and one at the Nevada Test Site by the Atomic Energy Commission. Five animals from each herd are being sacrificed twice a year (May and November). Rumen, fecal, and tissue samples are analyzed by gamma ray spectrometric methods by the Low-Level Counting Section. Tissue samples currently analyzed here are muscle, liver, and bone.

The present arrangement, which represents a considerable improvement, is that these samples are ashed at the University of Nevada by Dr. Verle Bohman, packed in standard polyethylene containers, and then shipped to LASL. This method considerably simplifies sample handling and calibration problems, and also increases sensitivity substantially.

Table I gives the results on liver and muscle samples from three animals of the NTS herd sacrificed May 1959. It should be noted that the Cs^{137} content is comparable with

TABLE 1. RADIOACTIVITY OF BEEF LIVER AND MUSCLE SAMPLES OF ANIMALS FROM THE NTS HERD SACRIFICED IN MAY 1959*

Tissue	NTS	LASL	Animal	Cs^{137} (μμc/g K)	Cs^{137} (μμc/kg)	Zn^{65} (μμc/kg)	g K/kg
Liver	B/31/59	841134	2-yr steer	61	282	195	4.6
	B/32/59	841133	1-yr steer	28	140	154	5.0
	B/33/59	841141	1-mo calf	58	180	97	3.1
Muscle	B/31/59	841149		122	342	107	2.8
	B/32/59	841142		76	229	77	3.0
	B/33/59	841135		112	503	153	4.5
Commercial Beef Loin and Rump (1958)				50	---	---	2.2
Commercial Hamburger, SRI (1959)				103	---	---	2.7

*The estimated error in NTS values is ± 10 per cent (K^{40}, Zn^{65}) and ± 15 per cent (Cs^{137}).

ordinary commercial beef. Humco I measurement of commercial beef loin and rump in 1958 gave 50 μμc/g K, and analysis of hamburger samples in 1959 for the Stanford Research Institute gave about 100 μμc/g K. Thus, proximity to atomic bomb tests had little or no effect on the Cs^{137} content of these animals.

Note that Zn^{65} is present in all of the 1959 samples in amounts one-half to one-third of the Cs^{137}. Figures 1 and 2 show gamma ray spectra of pooled muscle samples from animals sacrificed in May 1958 and 1959, respectively. $Zinc^{65}$ was not present in the May 1958 samples, but was present unquestionably in 1959. It made its first appearance in the November 1958 samples.

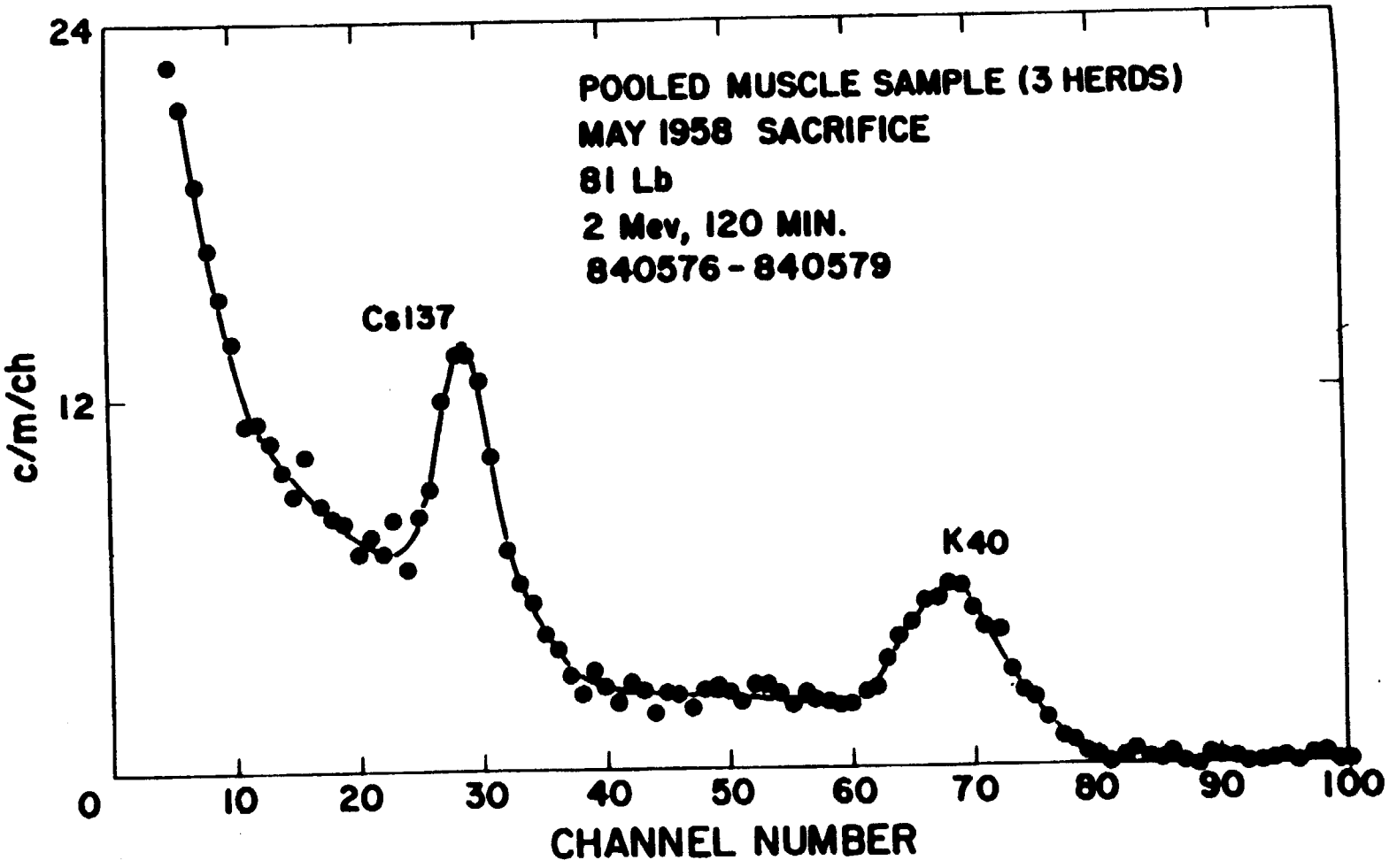


Fig. 1. Gamma ray spectra of pooled muscle samples from animals sacrificed in May 1958.

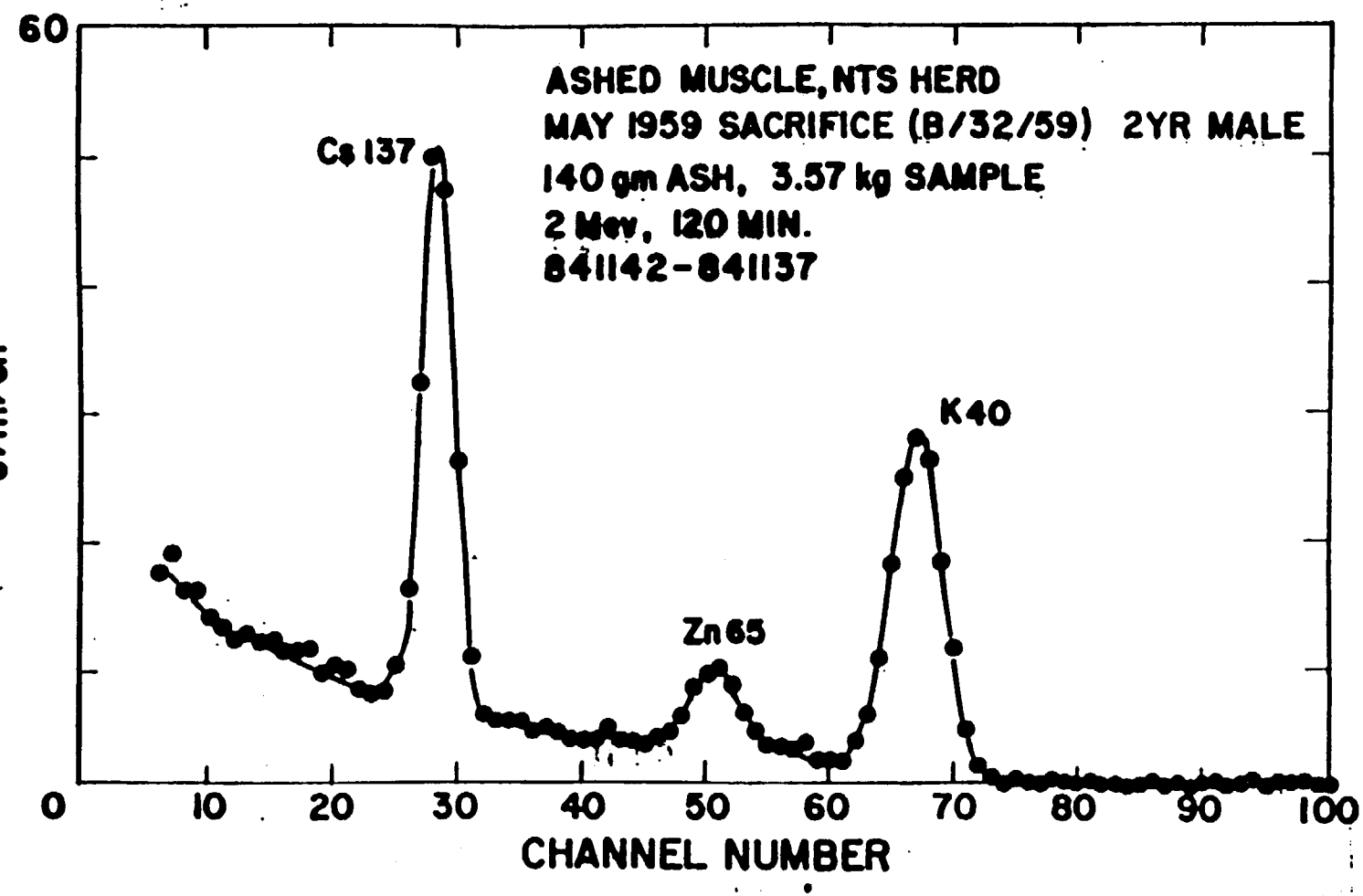


Fig. 2. Gamma ray spectra of pooled muscle samples from animals sacrificed in May 1959.

Radiation Dose Rates above the Atmosphere (M. A. Van Dilla, J. H. Larkins, J. D. Perrings, and R. D. Hiebert, Group P-1)

Introduction

In the spring of 1959, the Low-Level Counting Section was asked to cooperate with the Research Directorate of the Air Force Special Weapons Center at Kirtland Air Force Base, in an effort to measure biological dose rates in the Van Allen radiation belts. Our aid was desired in designing and building the radiation detector. Power supplies, telemetering, vehicle launching, etc., would be handled by Kirtland Air Force Base and other agencies. Decision was made to use a tissue-equivalent ion chamber, since this system seemed most suited to measure the poorly understood mixture of protons, electrons, and bremsstrahlung in the trapped radiation belts.

Initially, the vehicle envisioned was the solid-propellant Journeyman rocket to be launched from Wallop's Island, Virginia. The expected altitude was 2000 miles, and the magnetic latitude was such that penetration of the region between the inner belt and the horns of the outer belt would occur. The shot would be an altitude probe with no recovery of the payload anticipated. However, engine difficulties caused delay until spring 1960.

Arrangements are now underway to place the equipment on a Discoverer satellite. This program has as its objective a

recoverable capsule from a vehicle in a polar orbit at a couple of hundred miles altitude. The present (December 195 status of the vehicle problem is as follows: we hope for rides on Discoverers early in 1960 and on Journeymen later in 1960.

Results and Discussion

Development of the ion chamber has proceeded on schedul and in September 1959 the first unit was completed, tested, and calibrated. The ion chamber covers the range 0.01 to 100 r/hour; response is logarithmic with a maximum time constant of about 10 seconds. The dose range was selected o the following basis. Maximum dose rates estimated by Van Allen and others in the heart of the inner belt are roughly 100 r/hour. Minimum dose rates below the Van Allen belts and above the atmosphere at mid-latitudes are roughly 0.001 r/hour and are due to cosmic rays. Since the lower dose rates are of little biological significance, it was de- cided to cover the upper range. Four decades seemed practic for a logarithmic instrument (a linear response plus range switching was ruled out because of mechanical complica- tions).

Construction details of the chamber and electronics are shown in Fig. 1. The unit is made of Lucite, with a gas

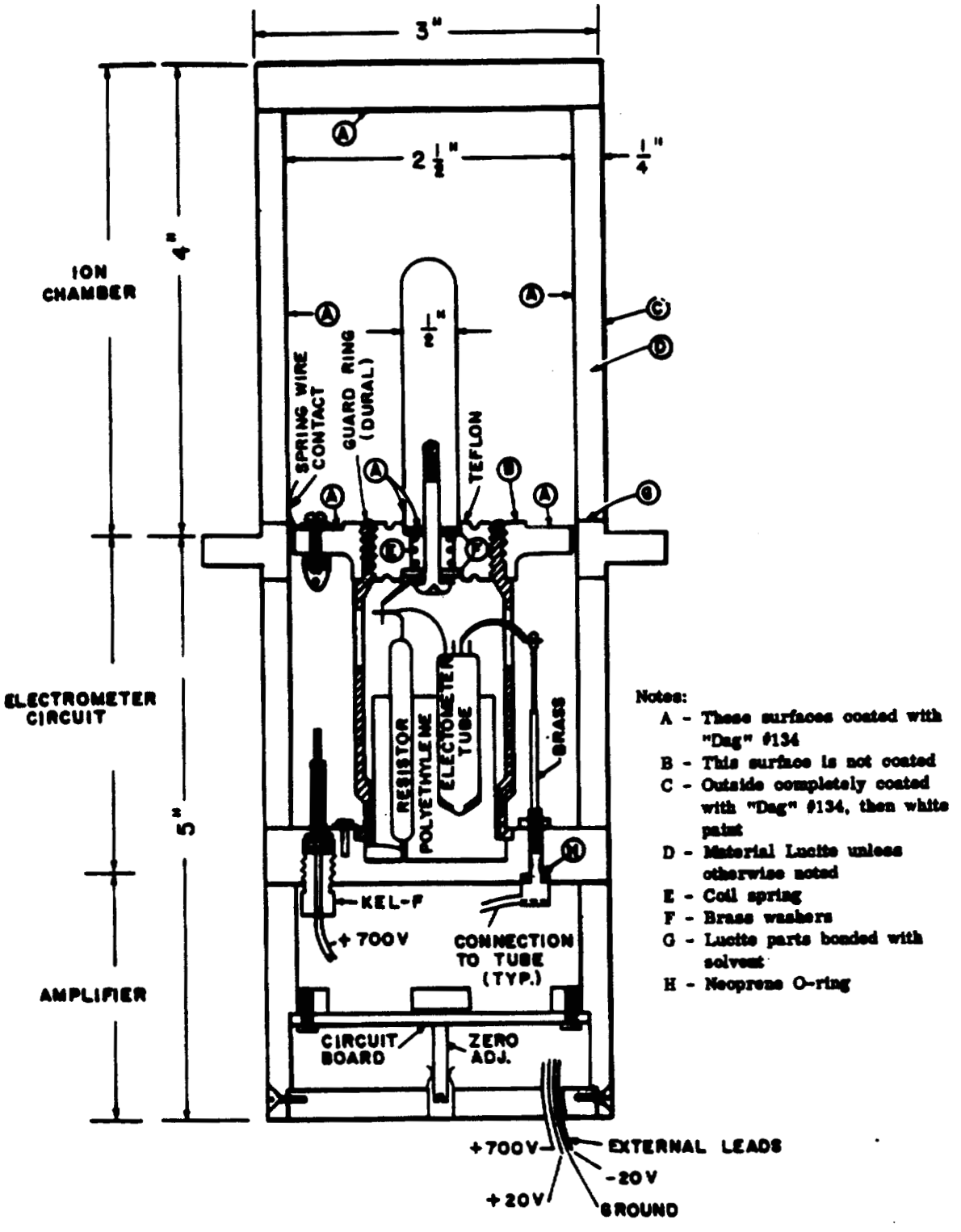


Fig. 1. Ion chamber for dose rate measurement in space.

volume of about 250 cc. The filling gas is air at essentiall atmospheric pressure. The wall thickness of 1/4 in. will exclude all protons softer than 25 Mev and all electrons softer than 1 Mev.

Lucite ($C_5H_8O_2$) was chosen because it resembles soft tissue insofar as the dose delivered by gamma rays, high ener electrons, and protons is concerned, and because it is a convenient and frequently used material for ion chamber construction. It has a density of 1.2 and consists of carbon (60 per cent), oxygen (32 per cent), and hydrogen (8 per cent). Soft tissue has similar density and is composed mainly of the same elements. The only difference is that the carbon-oxygen ratio is higher in Lucite, but this has negligible effect on energy dissipation and dose delivered by gamma rays and high energy electrons and protons. (This is not true for slow neutrons, but they are present to a negligible extent.)

The bottom half of the unit contains the electrometer tube, transistorized amplifier, and voltage regulator circuit. The output is 0 to 5 volts and drives the telemeter equipment in the vehicle. For the Discoverer shot, polarizing voltage across the chamber electrodes of 150 volts is supplied by a separate battery box containing five 30-volt hearing aid batteries.* When irradiated at a dose rate of 100 r/hr (Co^{60}

*Ever-Ready No. 507.

current saturation occurs at 100 volts.

The first two units were subjected to and successfully passed rigid environmental tests specified by Kirtland Air Force Base. When temperature tested from 40 to 80°F under irradiation, no detectable change in ion current occurred. When temperature tested from 80 to 140°F under irradiation, a 6 per cent change in response (in r/hour) was observed. The ion chamber was vacuum tested in a bell jar with source strapped to it; the pressure was reduced to about 0.05 atmosphere and held there for two hours with no ion current change. This test was repeated after chilling the chamber to 40°F; still no current change was observed. The unit was acceleration tested at Kirtland Air Force Base up to 100 g under irradiation. Acceleration had no effect on ion current. Vibration tests at 30 to 2000 cycles per second were performed under irradiation, and no effect on ion current was produced.

From the viewpoint of radiation hazards in space, the important physical quantities are radiation dose rate and linear energy transfer (LET) to both superficial and deep tissues. The present instrument will give the dose rate to the more superficial tissues. By increasing wall thickness, one can find out how the dose rate to the deeper tissues changes.

The ion chamber gives no information as to the fraction of the dose due to protons, electrons, and bremsstrahlung and is insensitive to differences in LET among these radiations. Not a great deal is known about the composition and energy spectra of the radiation in the two Van Allen belts, so it was judged advisable to concentrate attention on the dose rate itself.

Studies of the Feasibility of Designing a Lunar Gamma Ray Spectrometer (M. A. Van Dilla and E. C. Anderson)

Introduction

Approximately 18 months ago the Low-Level Counting Section was approached by Professor J. R. Arnold of the University of California at La Jolla regarding collaboration on a study of the feasibility of measuring the gamma ray emission of the lunar surface from a close lunar satellite. The request was made because of the Section's controlled low background counting facility and experience with low-level gamma ray spectrometry. Agreement was that the Section's staff would participate in experimental design of the detector, preliminary laboratory measurements, and analysis and interpretation of the final data. All building and packaging of the instrument would be done by industry on contract with the National Aeronautics and Space Administration. Installation in the vehicle and all telemetering operations would be the responsibility of the Jet Propulsion Laboratory. At present, the most likely rocket system for the experiment seems to be a Centaur in 1963, but a simplified preliminary experiment may be possible sooner. The purpose of the experiment is to provide information relevant to the composition and geologic history of the moon and to the radiation intensity at its

surface. During the past report period, some preliminary measurements of gamma activities of certain meteorite types and terrestrial minerals have been made and theoretical considerations initiated.

Results and Discussion

Theoretical predictions of concentrations of both natural and cosmic ray induced radioactivity have been made by Arnold, and the resulting gamma ray spectrum as seen by a 3 x 3 in. NaI crystal has been calculated by Harvey Israel of Group H-6. The results for the "thin source" approximation are shown in Figs. 1 and 2. From the point of view of theories of lunar formation, the K^{40} line at 1.45 Mev is of greatest interest. As a radiation hazard, the prominent series of neutron capture gamma rays in iron (4 to 8 Mev) must be evaluated.

Using the results for background in free space of a NaI crystal aboard the Russian rocket Mechta (1), it is estimated that the total background rate between 0.45 and 4.5 Mev would be about 5 times the total gamma ray signal from the moon in the same band, assuming the satellite to be close enough to the moon to give 25 per cent geometry (orbital distance 150 mi above the lunar surface). Since this background is presumably continuous, the gamma lines from the moon should have a more favorable ratio. Since the cosmic ray background in this energy region is probably due principally to local gamma

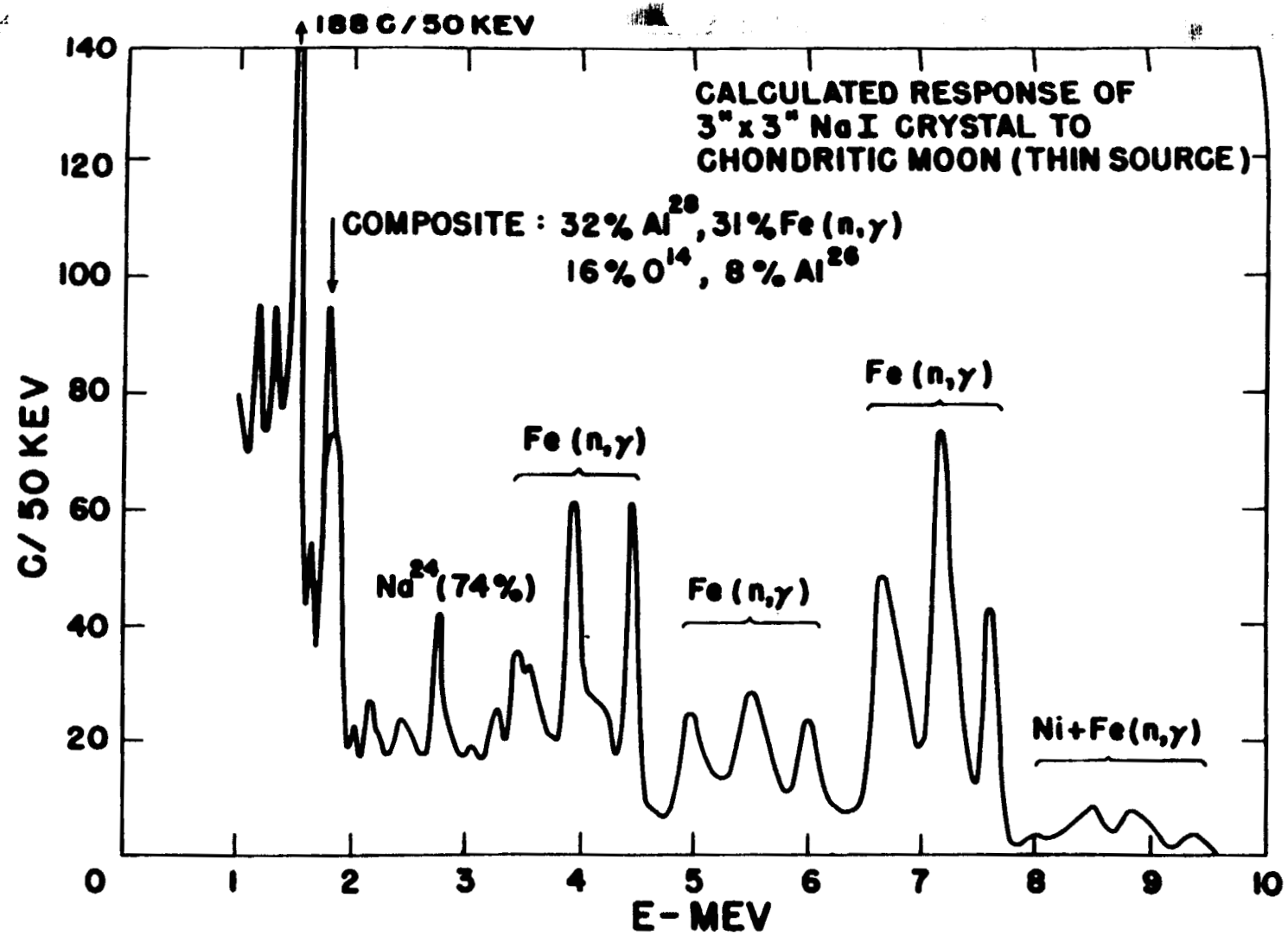


Fig. 1. Calculated response of 3 x 3 in. NaI crystal to chondritic moon (0 to 10 Mev).

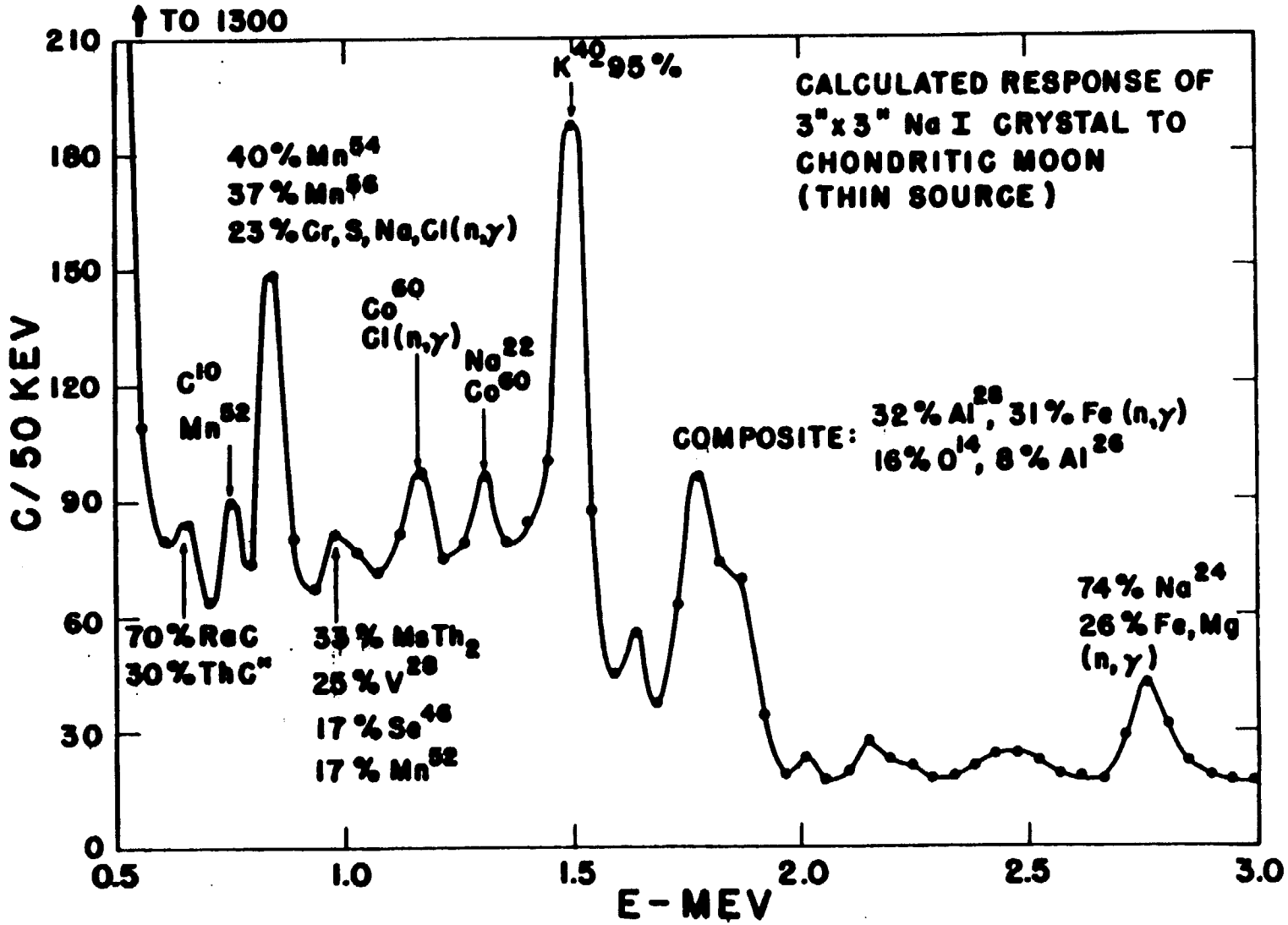


Fig. 2. Calculated response of 3 x 3 in. NaI crystal to chrondritic moon (0 to 10 Mev).

production, its magnitude will depend on the total mass of material near the detector. If necessary, this background could be reduced by extending the detector away from the satellite on a long boom.

The preceding spectra represent one of the least radioactive possibilities for the lunar surface (namely, a composition similar to that of chondritic meteorites). If extensive melting and recrystallization have occurred on the moon, then it may resemble the earth to the extent of having a surface mantle which is enriched in the natural radioactivities. Using the 8 x 4-in. NaI crystal spectrometer, measurements have been made of gamma ray activities of a number of materials which might be present on the lunar surface, including typical terrestrial igneous rocks such as dunites, gabbros, diorites, granites, and syenites, as well as several varieties of meteorites. Figures 3 and 4 show for comparison the spectra of a terrestrial biotite granite and a chondritic (stone) meteorite. Note that the K^{40} concentrations differ by a factor of about 100. Radium and thorium are prominent in the granite, but undetectable in the meteorite. Because of the large difference in K^{40} levels, even a comparatively crude experiment may distinguish between the alternative "hot" and "cold" models of lunar formation.

REFERENCE

(1) S. N. Vernov, A. E. Chudakov, P. V. Vakulov, and I. U. Ilogachev, Dokl. Acad. Nauk. S.S.S.R. 125, 304 (1959).

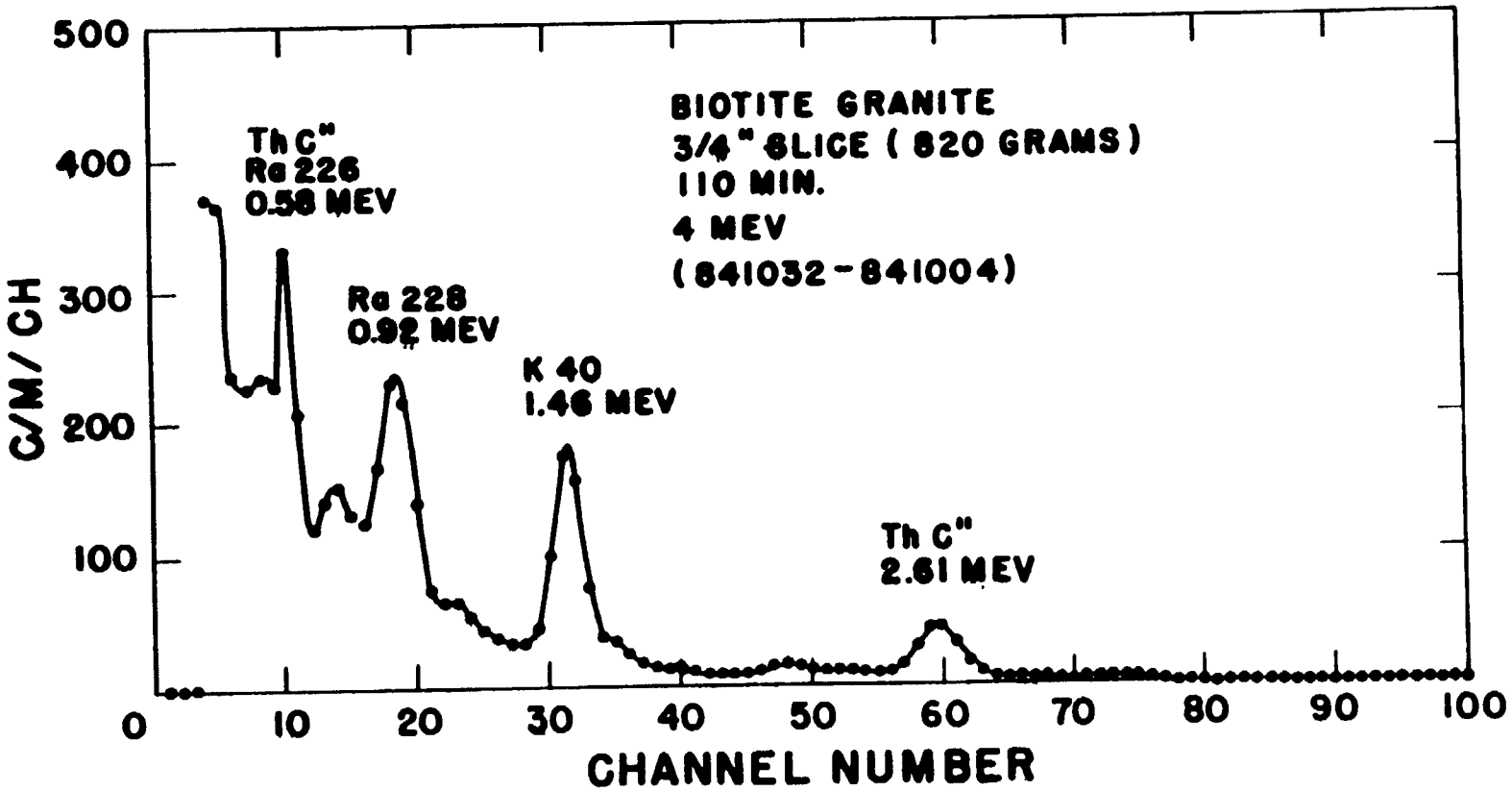


Fig. 3. Gamma ray spectrum of biotite granite.

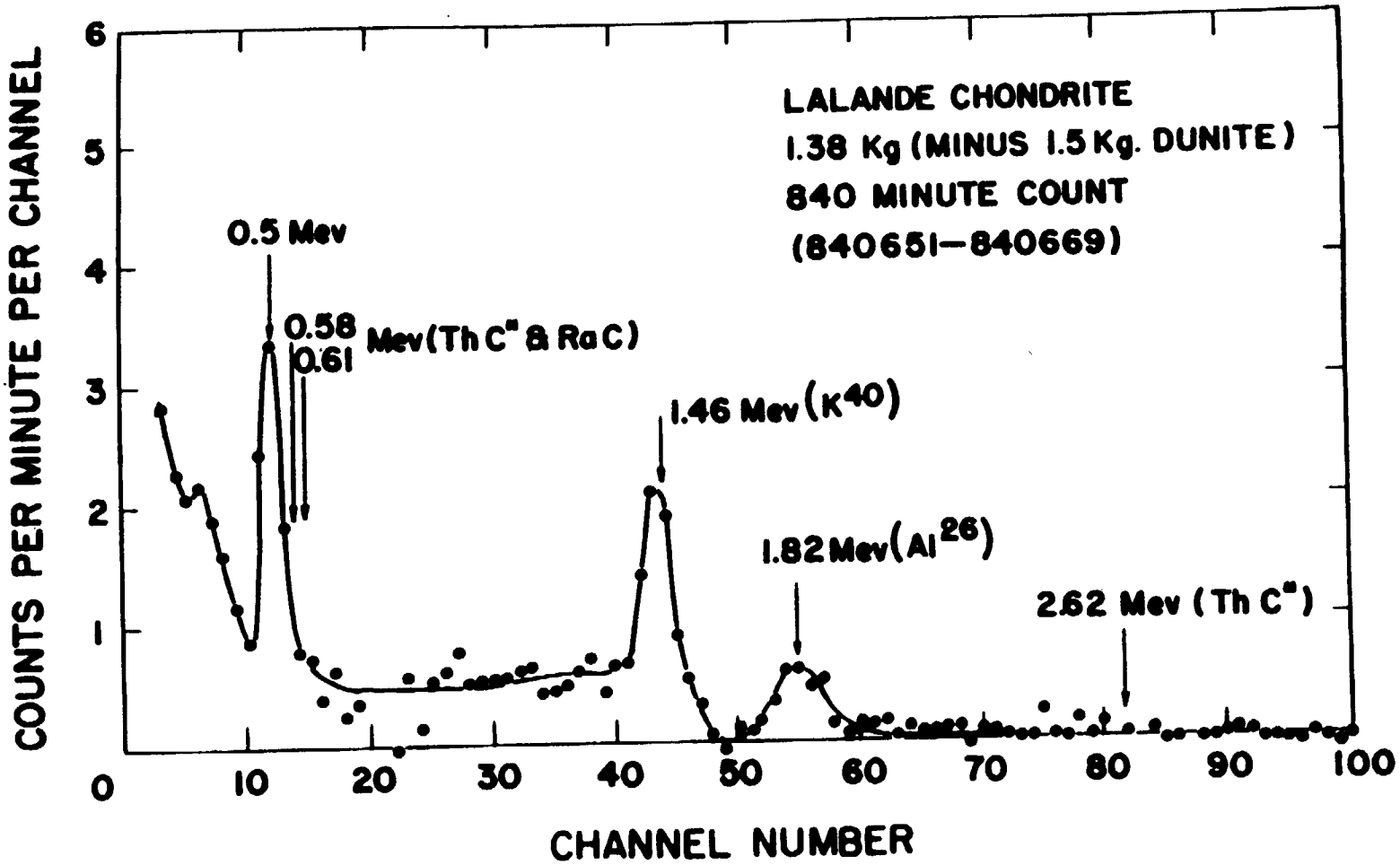


Fig. 4. Gamma ray spectrum of LaLande chondrite.

Development of Large Volume Liquid Scintillation Detectors

(R. L. Schuch, J. D. Perrings, and E. C. Anderson)

Introduction

A major project of the Low-Level Counting Section has been to explore the limitations and capabilities of large volume liquid scintillation detectors. The first 4π whole body counter built on this principle was Humco I (1), which has been in operation since July 1955. The second counter built ("Genco") was a 2π "walk-in" body counter using 14-in. photomultiplier tubes and a 6-in. liquid depth (2). This unit was displayed at the Second Geneva Conference on Peaceful Uses of Atomic Energy and is now in service at the U. S. Army Medical Research Unit in Landstuhl, Germany. On the basis of the very successful performance of the 2π counter, the decision was made to design and build an improved 4π unit (Humco II).

Humco II uses 24, 14-in. diameter photomultiplier tubes (DuMont K-1328) to maximize light collection efficiency. Scintillator thickness has been increased to 12 in. to improve counting efficiency, it is hoped without worsening energy resolution. The counter tank holds 415 gallons of scintillator (about 2600 pounds). An artist's drawing of the counter is shown in Fig. 1. Figure 2 is a photograph of the tank

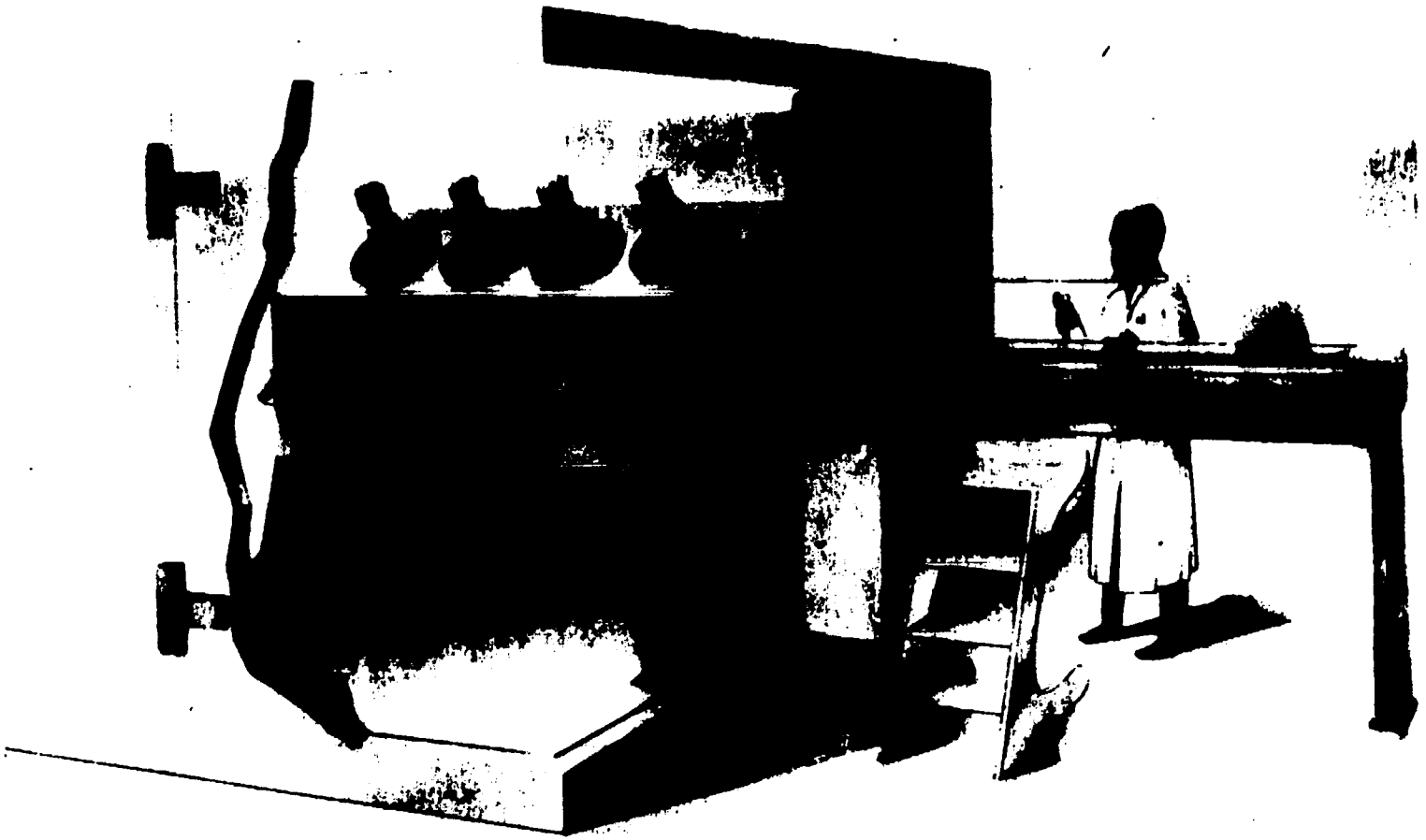


Fig. 1. Artist's drawing of Humco II.

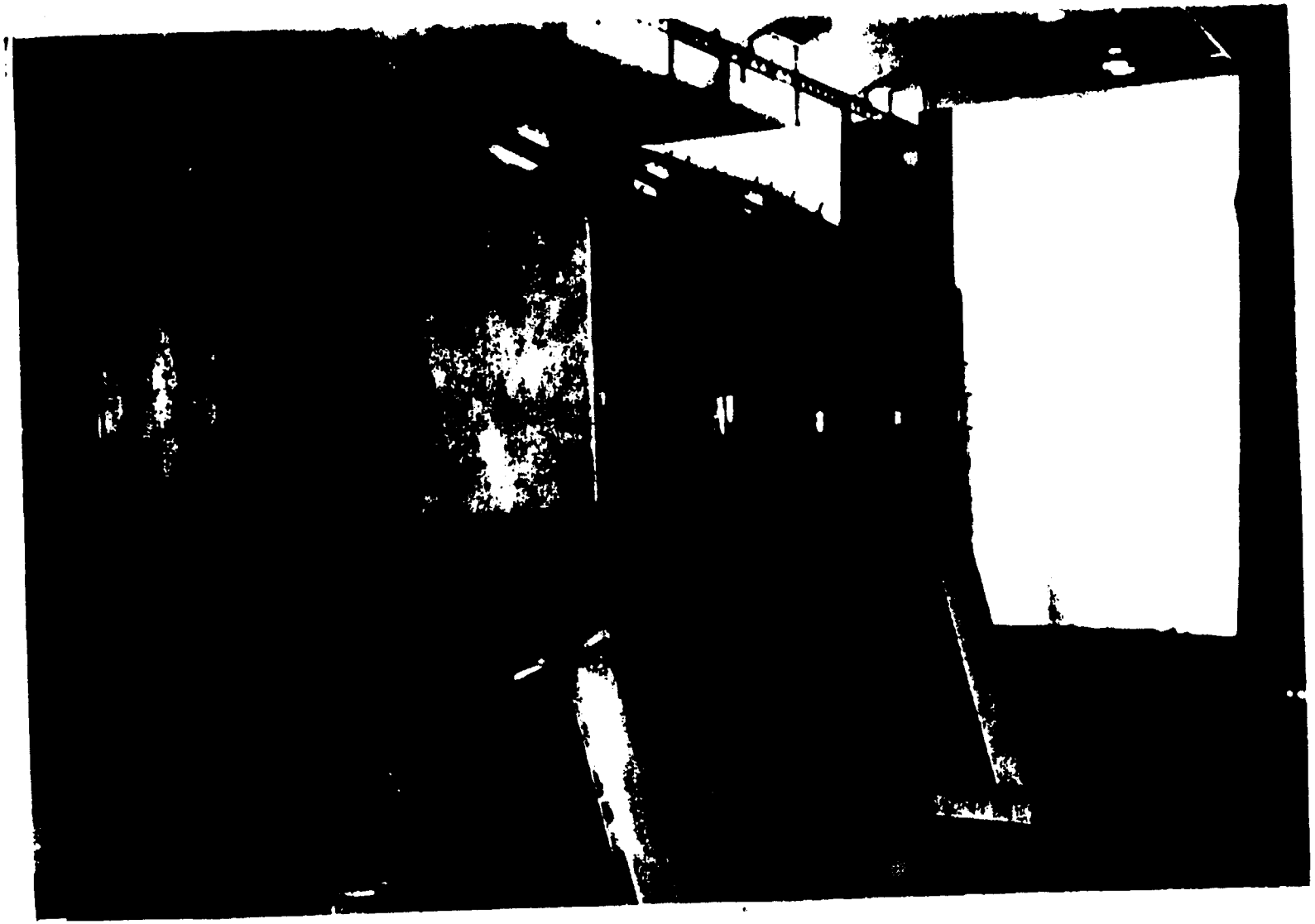


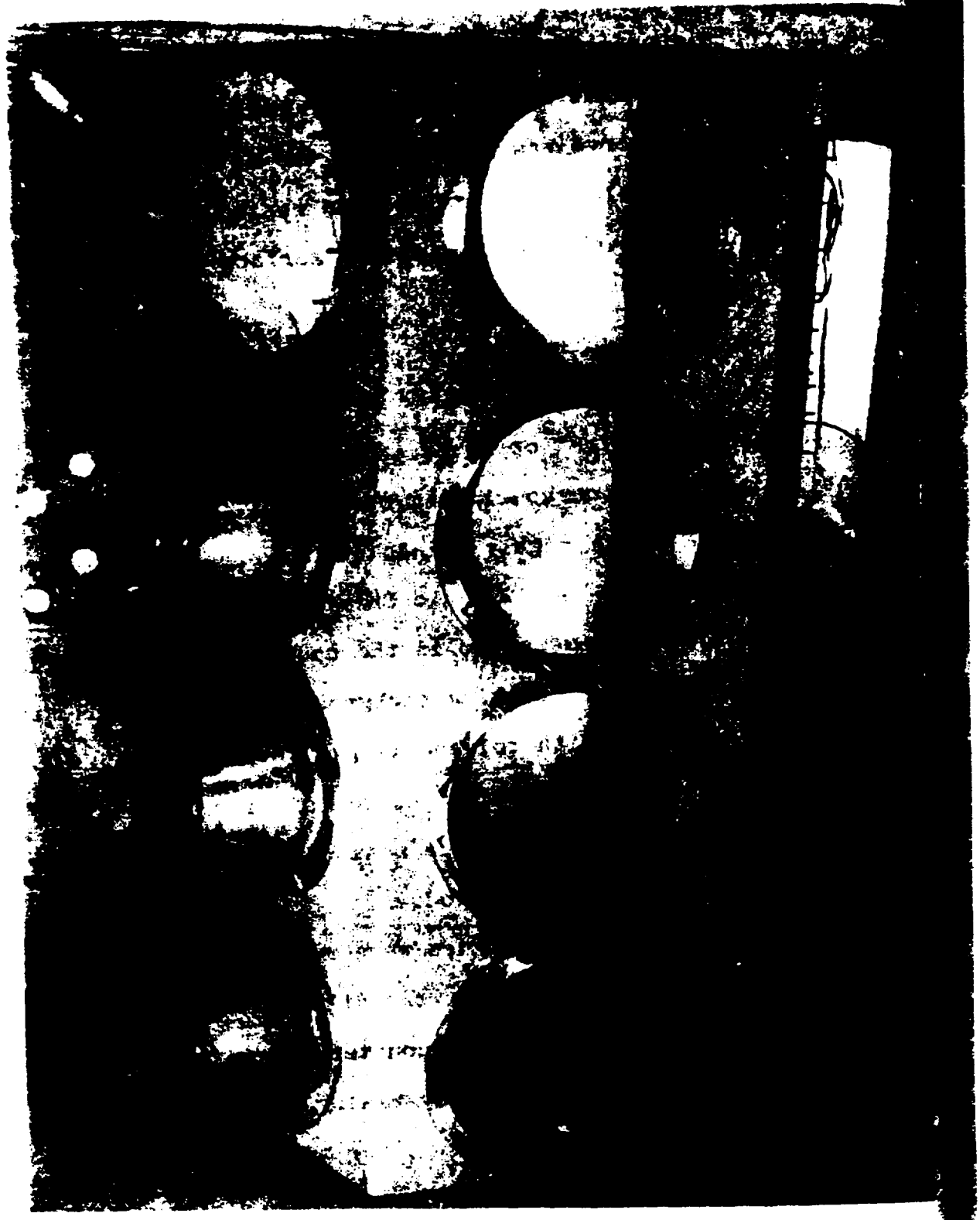
Fig. 2. Humco II in shop, before painting.

before painting and Fig. 3 a photograph during installation of the phototubes. The method of sealing the tube faces to the tank was the same as that used for Genco. A steel ring with an O-ring groove was cemented to the metal flange of the phototube envelope with an epoxide resin. The ring was then carefully attached to a machined surface on the tank with 16 studs.

Results and Discussion

Humco II was first operated on August 28, 1959, using a scintillation solution consisting of 5 g/l PPO and 0.05 g/l POPOP in ScinSol I (a highly alkalated benzene available from the Borden Company -- see Organic Chemistry Section's report). The first subject was measured in the counter with this filling on September 8. After checking background and standards, the counter was refilled with toluene (4 g/l terphenyl plus 0.05 g/l POPOP) on October 9. A number of minor leaks due to hairline cracks in the epoxide seals around the photo-tubes occurred during this period.

Tests of counter performance were repeated with the toluene filling. Figure 4 shows the differential background spectra obtained with the Geneva counter and with the two fillings of Humco II. The rapidly rising portions at the left ends of each of the curves are due to tube noise (as



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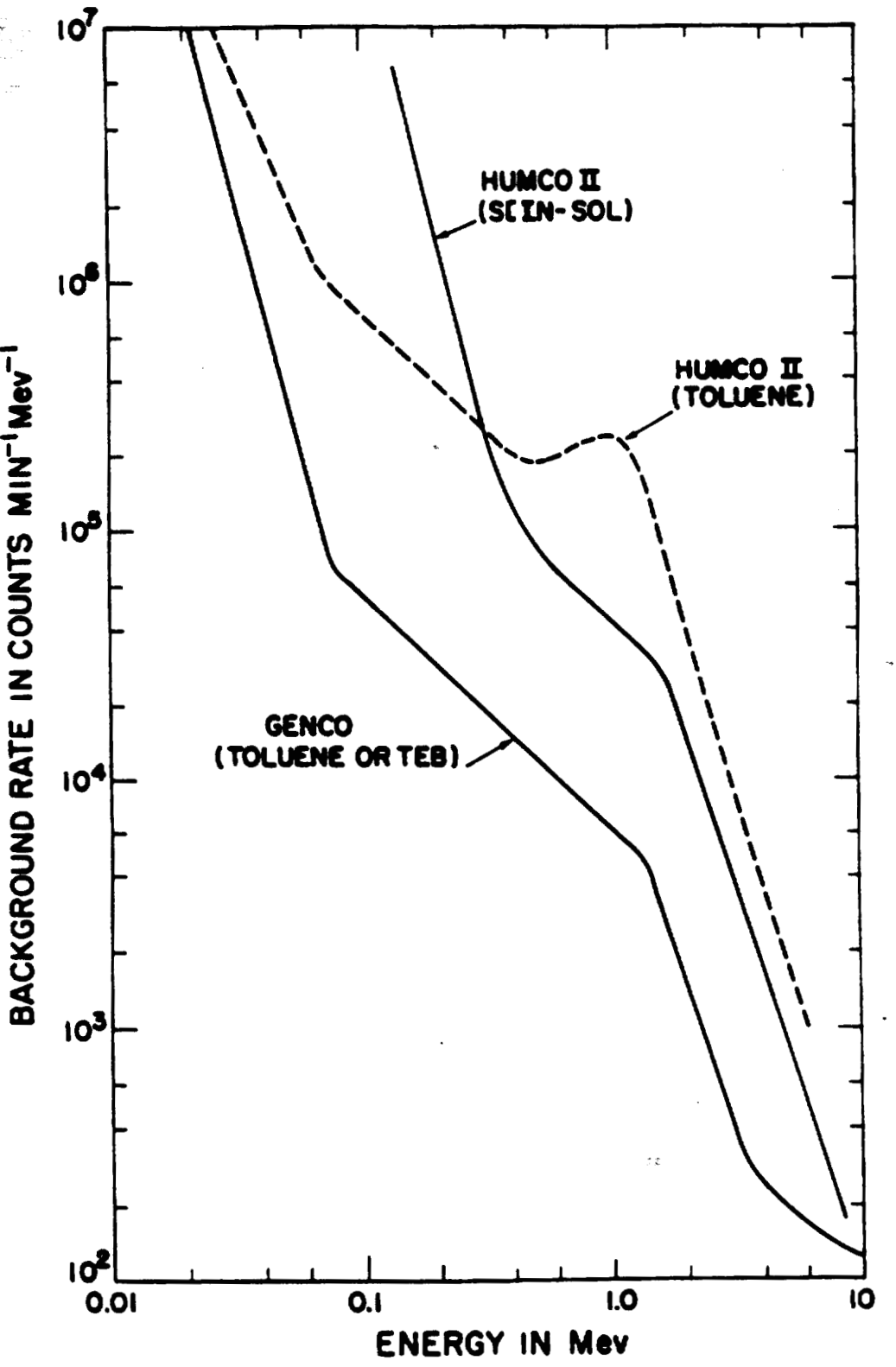


Fig. 4. Background spectra of Genco and Humco II.

can be demonstrated by a measurement with no solution in the counter). The intersections of these curves with the nearly linear portions of the spectra between 0.1 and 1.0 Mev set lower bounds to the energy ranges of the systems (unless coincidence operation is used). The greater the amount of scintillation light reaching the photocathodes, the lower the energy at which the tube noise interference begins. For Genco, this point is about 70 kev. Humco II with toluene gives a similar value, but with ScinSol I the value is 400 kev. Further study is being made by the Organic Chemistry Section of this and other nonvolatile solvents to find one that combines low flash point, low cost, and high light output. A 70-kev lower limit covers essentially all the useful range of a low Z scintillation and in particular permits bremsstrahlung counting. A higher cutoff seriously limits the versatility of the detector.

Figure 5 shows the differential energy spectra of Cs^{137}, Zn^{65}, and K^{40} as determined with the toluene filling. The resolutions (full-widths at half-heights) are 65, 57, and 42 percent, respectively; a variation nearly proportional to $\sqrt{E}$. The peak pulse heights are accurately proportional to the maximum Compton energy, giving energy calibrations of 28.9, 28.8, and 29.0 kev/v, and indicating that there is comparatively little multiple scattering even with the 12-in.

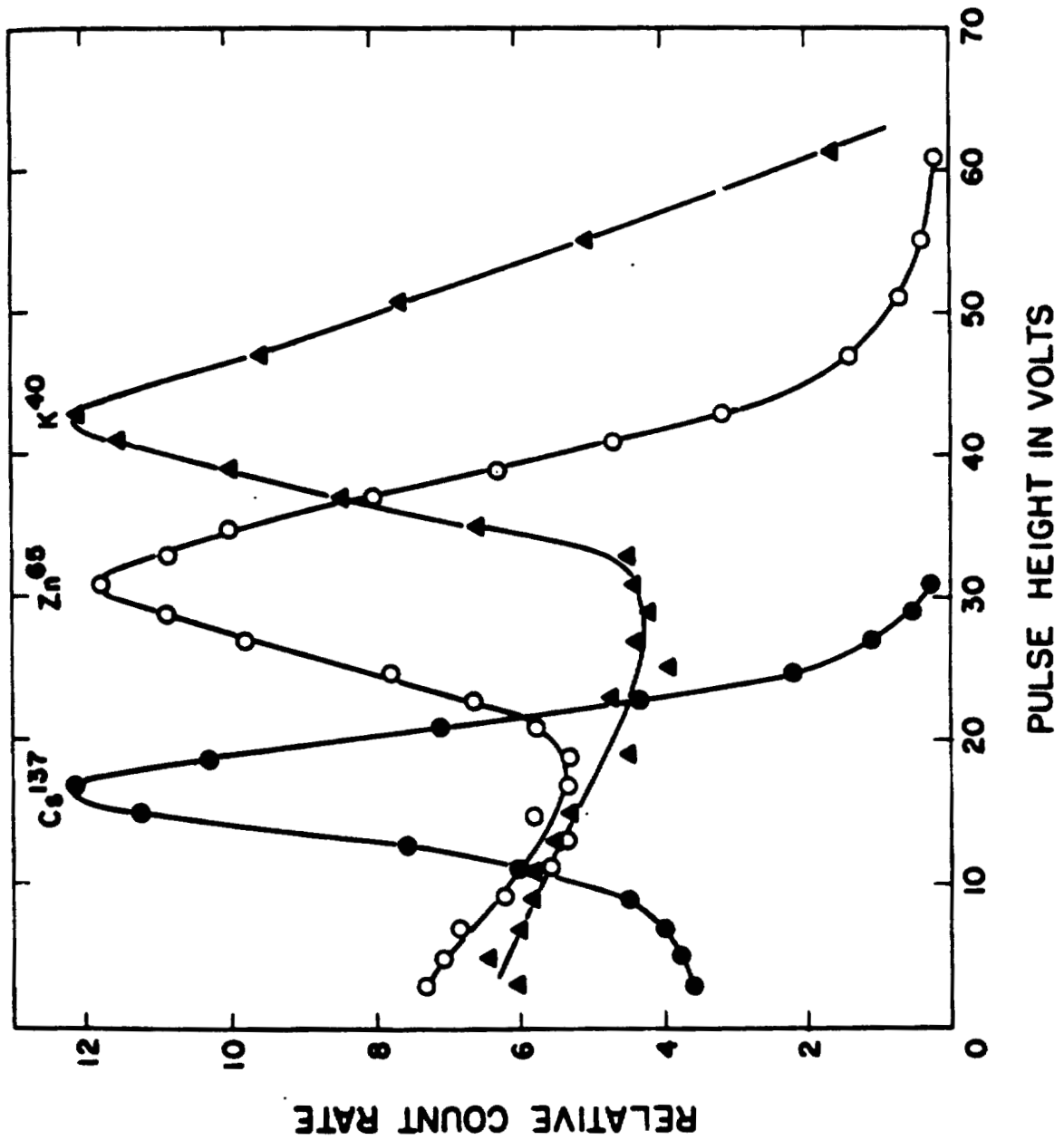


Fig. 5. Energy spectra of Humco II.

thickness of scintillator. Note that Zn^{65} and K^{40} can be resolved, a matter of some importance since the former nuclide is beginning to appear in foods and in people from certain areas.

On October 16, the tank was drained to permit repair of a leaking resin seal, and a cracked photomultiplier face was discovered. Subsequent measurements revealed that the machined surfaces of the tank against which the O-ring was made were seriously warped so that strains were being developed in the metal rings and transmitted to the glass. The counter is, therefore, shut down pending rectification of this difficulty.

Transistorized electronic circuits developed by the LASL electronics group for Humco II include direct transfer of output data to IBM punched cards. Testing of this equipment will be begun as soon as the counter is operational.

REFERENCES

(1) E. C. Anderson, R. L. Schuch, J. D. Perrings, and W. H. Langham, Los Alamos Scientific Laboratory Report LA-1717 (1955).

(2) E. C. Anderson, F. N. Hayes, and R. D. Hiebert, Nucleonics 16(8), 106 (1958).

Development of a Liquid Scintillation Counter for Small Animal Studies (R. L. Schuch)

Introduction

In 1953, a whole body small animal counter (LASAC I) was constructed on the liquid scintillation principle. The unit consisted of a well type arrangement in which the animal was placed in the center, surrounded by an annular space filled with scintillator solution viewed by six 2-in. photomultiplier tubes (1). The counter has proved extremely useful not only for metabolic studies of gamma emitting isotopes in rats and mice, but also for measuring the relative level of I^{131}, etc., in the circulating blood of people as a function of time. In the latter case, the individual's forearm and hand were placed in the well and the output from the counter fed into a rate meter and recorder. The utility of this unit has prompted studies to develop an improved model.

Results and Discussion

Further information was obtained on the relative counting efficiency for a point source and live mice at various positions along the central axis of the counter. The results obtained varied little from the calculated geometrical end loss.

As a result of the information obtained, a final design (LASAC III) is now under construction. The maximum length of the new detector will be 12 in., and the diameter of the well and number of photomultipliers will remain the same. The photomultiplier tubes will be mounted directly into the liquid. This should improve light output 10 to 20 per cent, and the natural background should be reduced by an order of magnitude due to over-all reduction in size. The counter is designed with both ends of the counting well open to accommodate a larger number of experiments.

Preliminary tests have been completed on a prototype of a more efficient and compact counter (LASAC II). This unit consists of a tank 12-1/2 in. in diameter and 12 in. long with seven 3-in. diameter DuMont photomultiplier tubes fitted on one end. A central counting well 4-1/4 in. in diameter is surrounded by an annular ring of scintillator solution 4 in. thick (Fig. 1). The photomultiplier tubes are coupled to an interface of window glass disks and are held in place by molded RTV 501 silastic.

Photomultiplier tubes were individually balanced for pulse height output and their signal outputs were connected in parallel (Fig. 2). The tank is filled with 15 liters of scintillator solution containing 4 g/l terphenyl plus 0.05 g/l POPOP in toluene. The signal output is fed into a

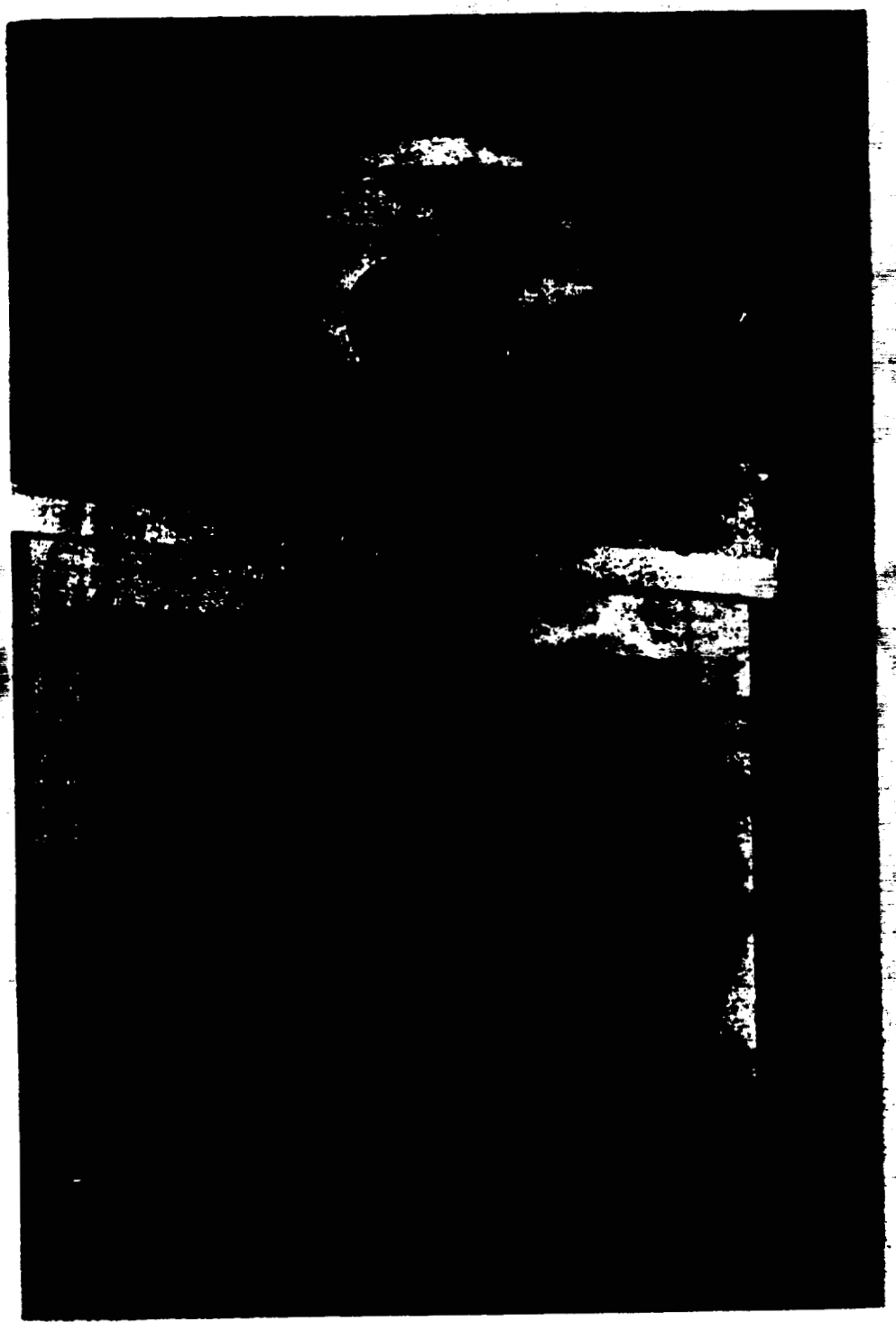


Fig. 1. The Los Alamos Small Animal Counter (LASAC II).

Fig. 2. Photomultiplier tube assembly for LASAC II.

100-channel analyzer. Spectra were run on sources of Co^{60}, Cs^{137}, Ce^{144}, and Cr^{51} centrally located in the counter well. The data obtained with a Cs^{137} source (which was the isotope most thoroughly studied) are summarized in Table 1, and these are compared with the same measurements made on LASAC I.

TABLE 1. COMPARISON OF Cs^{137} MEASUREMENTS MADE ON TWO MODELS OF A SMALL ANIMAL LIQUID SCINTILLATION COUNTER

Model	Isotope	Signal/Noise	Counting Efficiency	Lower Limit (kev)	
				E_γ	Compton Edge
LASAC I	Cs^{137}	4	0.53	400	133
LASAC II	Cs^{137}	9.3	0.40	150	52

REFERENCE

(1) K. T. Woodward, C. R. Richmond, and W. Langham, Proceedings of the Health Physics Society, p. 79 (1956).

Feasibility Studies of an Adjustable Plastic Scintillator Counting System (M. A. Van Dilla and R. L. Schuch)

Introduction

A scintillation counter consisting of a flexible system of individual cylinders of plastic scintillator has been constructed and certain performance characteristics studied. The primary purpose of this work was to develop a sensitive gamma ray detector with an adjustable arrangement and sample size capable of obtaining maximum energy sensitivity and resolution from a plastic scintillator system. Some potential applications of such a unit may be metabolic studies of gamma emitting isotopes in animals, measurement of rate of buildup of very low levels of activity on adsorption and purification columns, measurement of rate of flow of very low specific activity solutions through piping systems, uniformity of radioactively impregnated films, etc.

Construction

Cylinders of a commercial plastic scintillator* were selected for initial experiments for several reasons: high quality and availability, ease with which photomultipliers could be coupled to the end faces, and simplicity of unit

*Pilot Chemicals, Inc., Watertown, Mass.

construction. Each unit consisted of a 3-in. diameter cylinder 12 in. in length with a 3-in. photomultiplier tube fitted on one or both ends. The cylinders were wrapped individually with aluminum foil for reflectance and with black masking tape for light exclusion. The over-all length of a two-tube unit (frequently referred to as a "log") was 28 in. A single-tube unit measured 20 in. in length. In the case of the two-tube log, the photomultipliers had to be carefully balanced for pulse height output. As many logs as required can be arranged into a multiple unit around the sample and the outputs fed in parallel into a suitable electronic system for amplifying and recording pulses.

Observations and Discussion

Preliminary measurements were made with a multiple-unit of five two-tube logs fitted around a metal cylinder 4-1/4 in. in diameter (Fig. 1). Tube balancing and response of each log to a given energy peak were accomplished through use of a high voltage distribution panel and a multichannel analyzer. The output of each log was fed to the input of the analyzer, and by proper adjustment of the high voltage to each tube the pulse heights could be matched very accurately. The pulse height is increased by a factor of about 2, when both tubes view the scintillator together (Fig. 2). A Cs^{137}

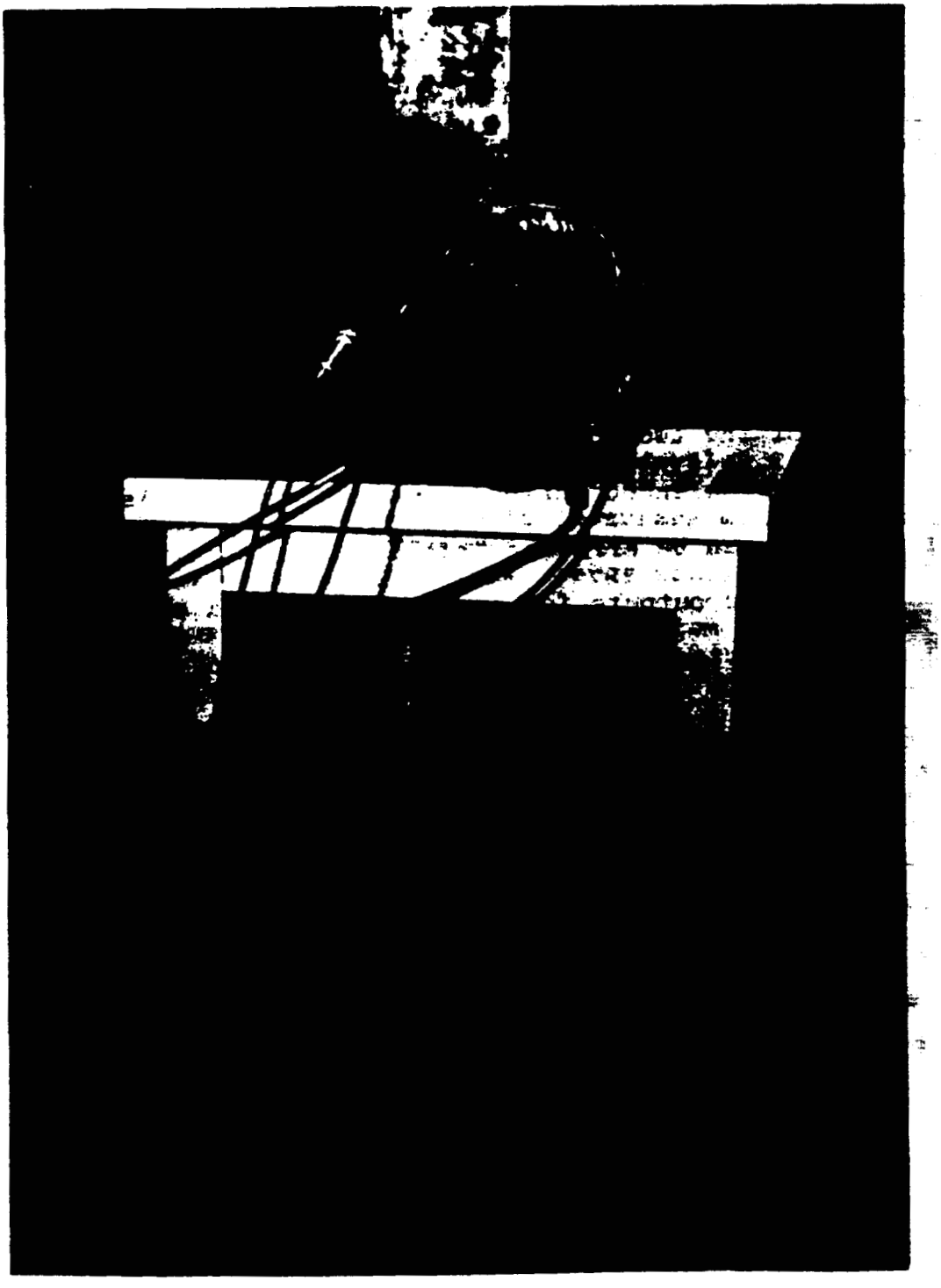


Fig. 1. Five-unit log roll with high voltage distributio
panel.

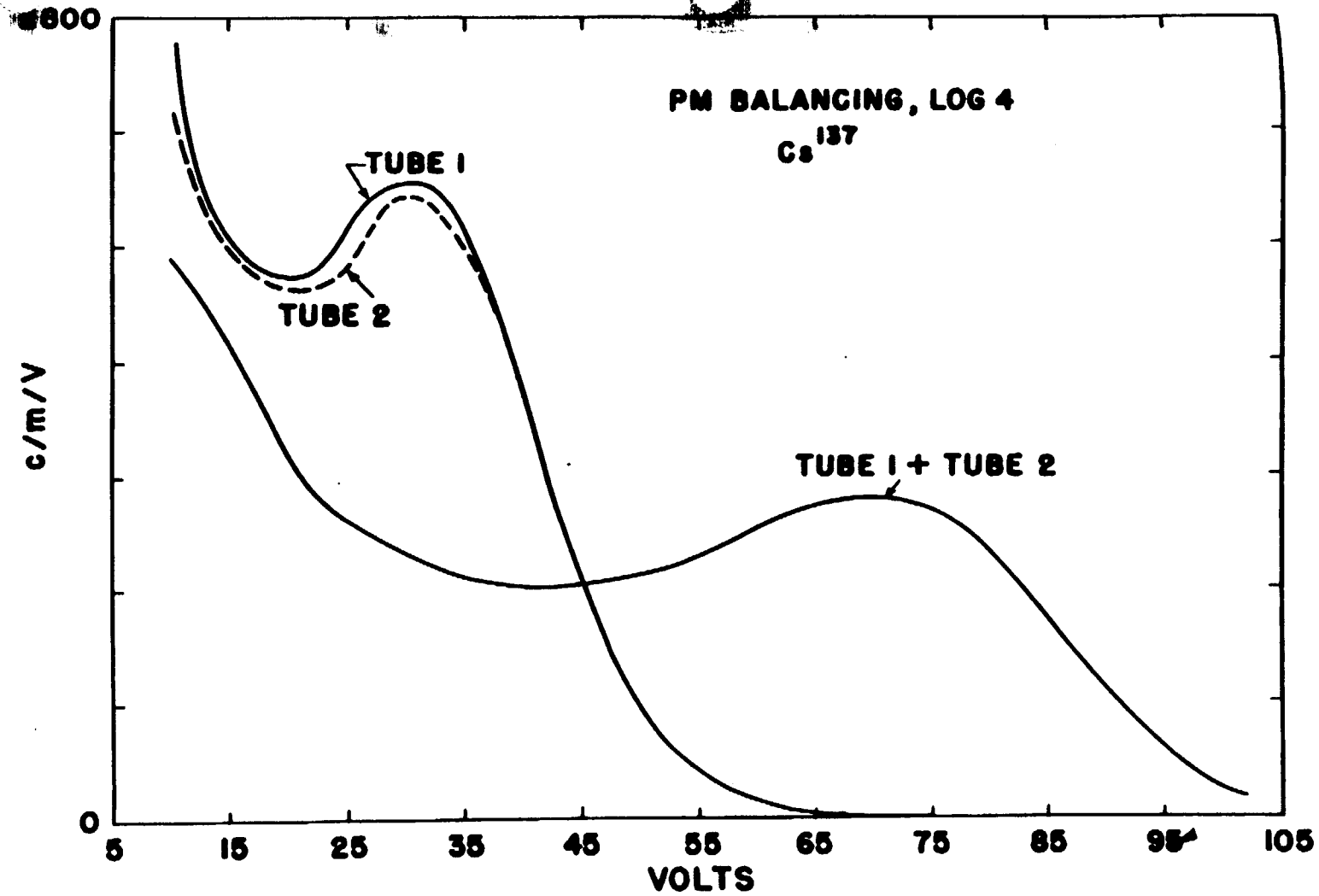


Fig. 2. Comparative pulse heights for Cs^{137} with 1-, 2-, and 1 plus 2-tube combinations.

source (gamma ray energy 0.667 Mev) was centrally located along the axis of the arrangement. This gave a counting-efficiency of 20 per cent, with the lower gate set at 100 k. Half-resolution with the five-unit system was 26 per cent.

Further studies were carried out to determine the combination of log length, optical coupling, light reflecto etc., that would yield the highest efficiency and sensitivi for a given gamma ray energy. Individual experiments were made with logs of 9-in. and 12-in. units and with one- and two-photomultiplier tubes viewing the log. When only one tube was used, the opposite face of the log was covered with aluminum foil to improve light collection. In order t study effect of counting efficiency versus cylinder length, a pencil source of Cs^{137} gamma rays normal to the axis of t log was placed at various distances from the photomultiplier tubes. Very little effect was observed with the 9-in. unit and only a 4 per cent loss was noted with the source on the extreme end of the 12-in. cylinder. Some of the plastic cylinders were made of two pieces bonded together. These u showed a greater variation in pulse height when compar to solid cast log.

Investigations were carried out to determine the lower limit of gamma ray energy that might be measured with one- and two-photomultipliers. Studies included units 9 in. and

12 in. in length. Bonded pieces were compared to solid cast, first individually, and finally in combination as a five-log multiple unit. The best results were obtained with the 9-in. cylinders. In all cases, two tubes were better than one. These conclusions applied also when signal to noise ratios and half-width resolutions of the various combinations were used as criteria (Table 1).

Energy Resolution.--Energy resolution is limited in organic scintillators by the broad peak resulting from Compton interactions in the low atomic number constituents. With the best tube-log combination (Table 1, 9-in. long, [illegible]tube unit), a half-width resolution of 15.5 per cent was obtained for Cs^{137} gamma rays (Fig. 3).

Effect of Light Reflectors.--In terms of total reflectivity, it is well known that a diffuse reflector (e.g., dry magnesium oxide powder) is superior to a specular reflector such as metal foil. A reflector test chamber was constructed to study the effect of various reflectors in the log unit geometry. The chamber was a light-tight aluminum cylinder with spacers, caps, and plugs to accommodate the various combinations of logs and reflectors. A photomultiplier tube was coupled to the plastic log and placed into the test chamber. For specular reflectance tests, aluminum foil was fitted around the plastic cylinder. When magnesium oxide

TABLE 1. PERFORMANCE OF VARIOUS CYLINDER-PHOTOTUBE COMBINATIONS WHEN TESTED WITH A Cs^{137} GAMMA RAY SOURCE

Description	PM Tube	Cs Peak / Noise	Half-Width Resolution (per cent)	Lower Limit (kev)	
				E_γ (kev)	Compton Energy
3" dia. x 12" long	One end	7.8	26	150	60
Bonded	Both ends	10.8	20.7	125	44
3" dia. x 12" long	One end	8.0	23	150	59
Solid	Both ends	13.0	20.5	115	37
3" dia. x 9" long	One end	11.3	18.0	120	42
Solid	Both ends	16.6	17.7	100	29
5-log system, 3" dia. x 12" long Solid and Bonded	Both ends	11.4	26	120	42

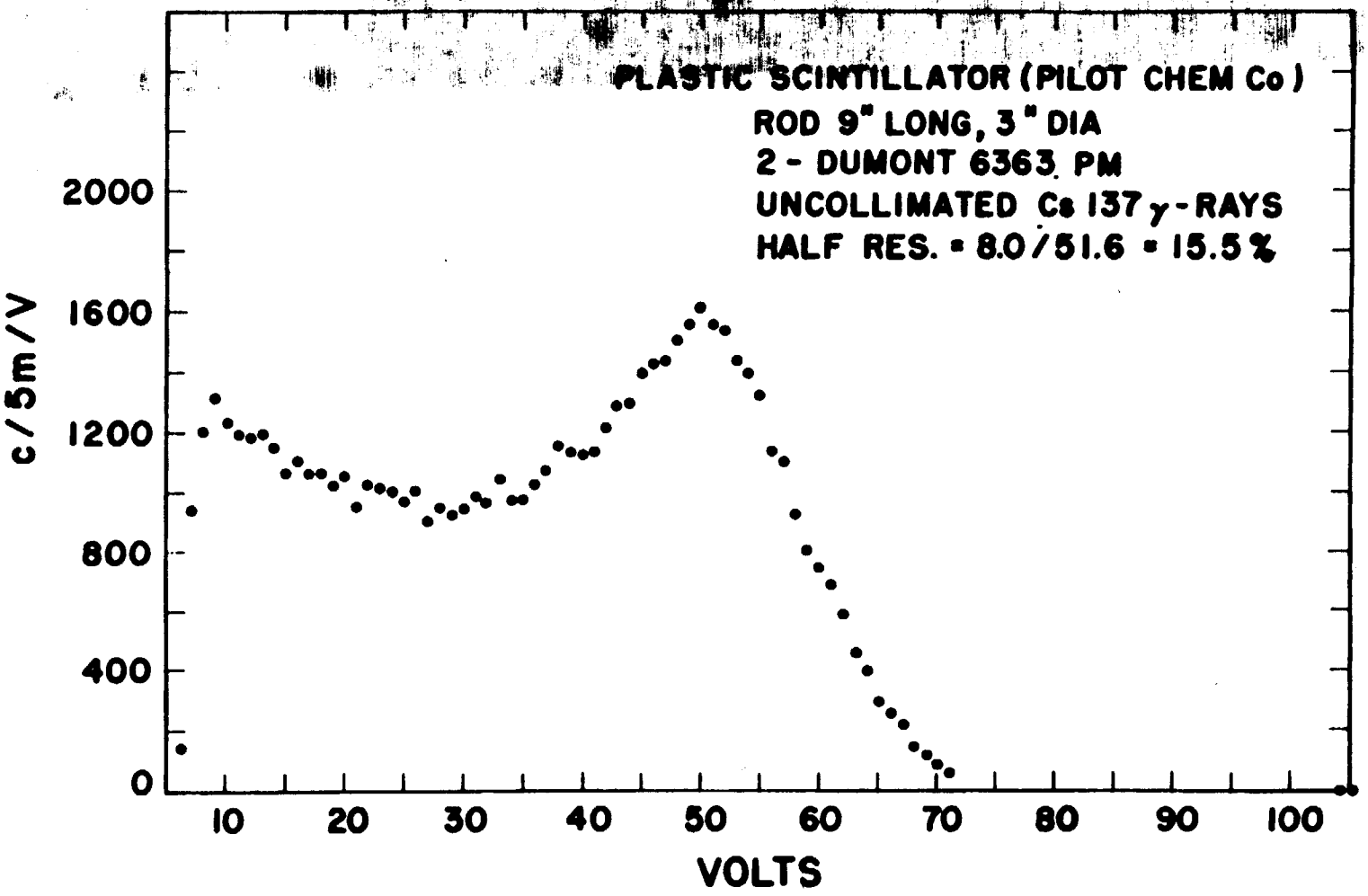


Fig. 3. Half-width resolution obtained for Cs^{137} gamma rays.

was used, the powder was poured and tamped in the annular space between the log and chamber wall. A comparison of light reflectors is shown in Table 2. Fifty per cent of the total gain in light collection is accomplished by placing the reflector on the end opposite the tube. A complete surface of magnesium oxide increased the pulse height by 68 per cent, and in all cases was 10 to 15 per cent better than aluminum foil.

TABLE 2. EFFECT OF VARIOUS REFLECTOR ARRANGEMENTS ON PULSE HEIGHT OF PLASTIC SCINTILLATOR CYLINDER (12 in. long x 3 in. diameter, one 3-in. photomultiplier tube)

	Pulse Height Ratio to no Reflector Same Scintillator		
Reflector	End Only	End and Sides	Ratio, E + S/
Plastic Scintillator[a]			
Al foil	1.18	1.43	1.21
MgO	1.32	1.68	1.27
Liquid Scintillator[b]			
Al foil inside	1.16	0.80	0.68
Al foil outside	1.28	1.80	1.41
MgO outside	1.32	2.00	1.52

[a] Pilot Chemicals, Inc., Watertown, Mass. Scintillator "B."

[b] Four g/l terphenyl plus 0.05 g/l α-NOPON in isopropylbiphenyl.

Comparison of Plastic and Liquid.--Similar tests with a cylinder filled with liquid scintillator were made, but direct comparisons of the two systems could not be accomplished because of dissimilar reflector surface application. An optimum liquid system, however, should produce the same result as the best plastic combination. The liquid cylinder consisted of a hollow Lucite tube filled with a solution of 4 g/l terphenyl plus 0.05 g/l α-NOPON in monoisopropylbiphenyl. The photomultiplier tube dipped directly into the liquid and fitted into the test chamber. Magnesium oxide and aluminum foil were placed on the inside of the cylinder for comparison. Again, e magnesium oxide increased the output by a factor of 2. Since the walls of the Lucite container were much poorer than the surface of the plastic for total internal reflection, there was a greater difference in pulse heights with the reflector at the end only, and end plus sides. With the aluminum inside the Lucite cylinder, directly in the liquid, a greater light loss was noted. The Lucite, although a poor internal reflector, was 20 per cent better than the foil specular reflector. The results are summarized in Table 2.

Optical Coupling.--Optical coupling between the photomultiplier tubes and the end of the plastic cylinder was made by applying a highly transparent film of petrolatum or silicon oil to the end of the plastic. There was little difference tween plain white petrolatum and the more exotic silicon

materials. In a liquid system, it may prove more practical to use a window interface. Disks of quartz, window glass, and Pyrex 1/8 in. thick were cut to fit over the 3-in. diameter face of a plastic cylinder. The disks were coupled to the plastic and to the photomultiplier tube with petrolatum, and the pulse height output (from a Cs^{137} source) of each was measured. The greatest variation noted was a 33 per cent reduction when Pyrex was used as the interface. Quartz and window glass, in turn, reduced the pulse height only about 10 per cent. A summary of studies with different optical couplings is shown in Fig. 4.

Conclusions

A counting system based on the principle of individual cylinders of plastic scintillator can be arranged in a variety of geometries, depending on the requirements of the user. Except for the shielding problem, such systems are relatively easy to design and construct. With a two-tube system, the lower limit of measurement is reduced to 100 kev gamma ray energy; lower levels may be obtained by proper selection of photomultiplier tubes. The absolute counting efficiency for Cs^{137} (667 kev gamma rays) with a 3-in. diameter, 9-in. long cylinder is about 34 per cent. That is to say, that 34 per cent of the cesium gamma rays entering the plastic scintillator are counted, disregarding geometrical and end losses.

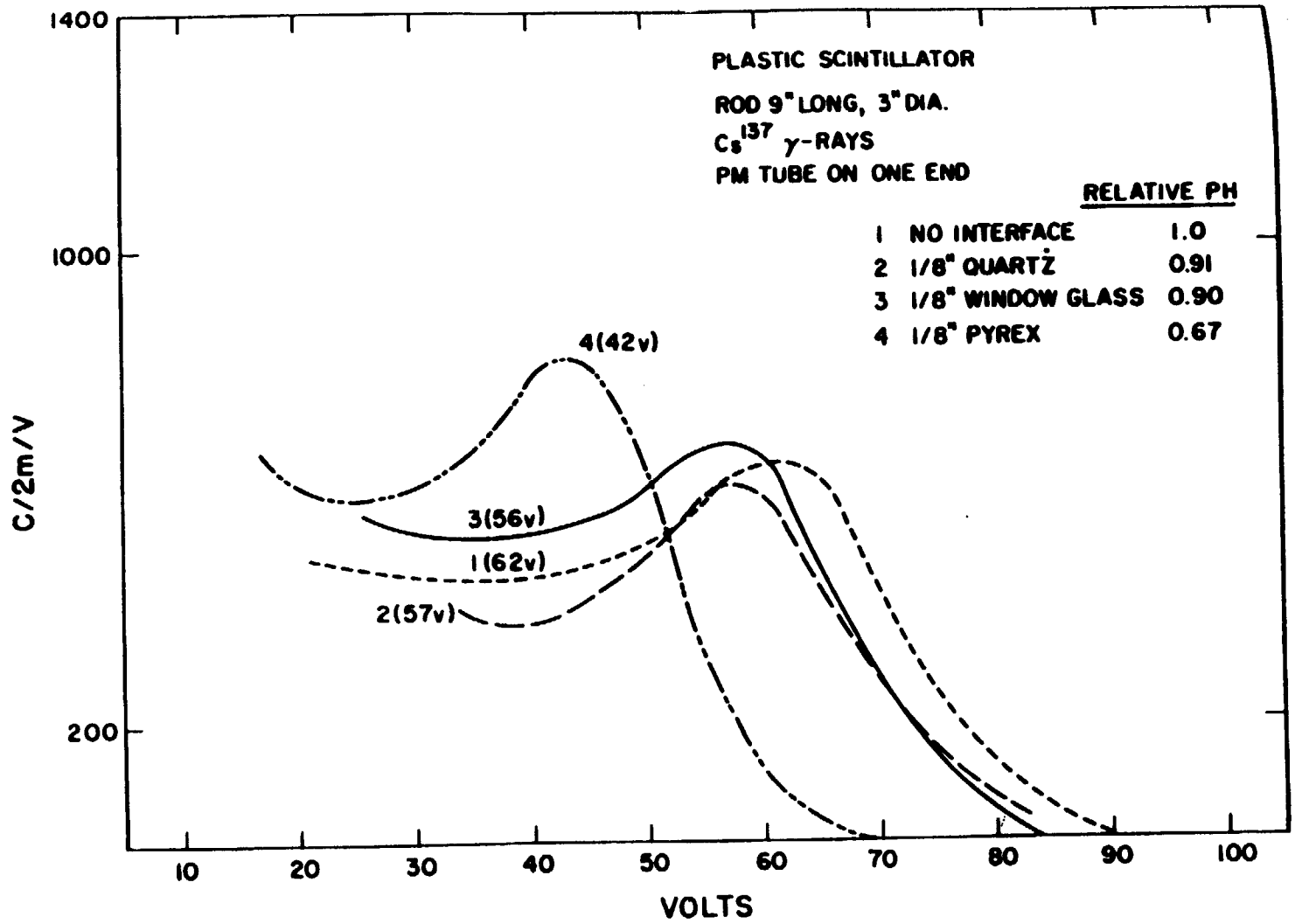


Fig. 4. Results obtained with various optical couplings.

LOW-LEVEL COUNTING SECTION PUBLICATIONS

(1) E. C. Anderson and W. H. Langham, Average Potassium Concentration of the Human Body as a Function of Age, Science 130, 714 (1959).

(2) E. C. Anderson, Applications of Natural Gamma Activity Measurements to Meat, Food Res. 24, 605 (1959).

(3) W. Langham and E. C. Anderson, Biospheric Contamination from Nuclear Weapons Tests through 1958. In: Hearings before the Special Subcommittee on Radiation of the Joint Committee on Atomic Energy, Congress of the United States, Eighty-sixth Congress, First Session on Fallout from Nuclear Weapons Tests (May 5-8, 1959), Vol. 2, 1061 (1959).

(4) W. H. Langham and E. C. Anderson, Cs^{137} Biospheric Contamination from Nuclear Weapons Tests, Health Phys. 2, 30 (1959).

(5) W. H. Langham, Implications of Space Radiations in Manned Space Flight, Aerospace Med. 30, 410 (1959).

(6) R. L. Schuch, D. L. Williams, and F. N. Hayes, Wide Range Gamma-Neutron Monitor, ISA Proc. 2, 95 (1959).

(7) M. A. Van Dilla, Large Crystal Counting, Nucleonics 17(11), 150 (1959).

MANUSCRIPTS SUBMITTED AND ACCEPTED

(1) M. A. Van Dilla and E. C. Anderson, Personnel Monitoring with Large Liquid Scintillation Counters, Armed Forces Med. J.

(2) M. A. Van Dilla, J. R. Arnold, and E. C. Anderson, Natural and Cosmic-ray Induced Radioactivities in Meteorites, Geochimica et Cosmochimica Acta.

(3) M. A. Van Dilla and M. J. Engelke, Zinc^{65} in Cyclotron Workers, Science.

(4) M. A. Van Dilla, Zinc^{65} and Zirconium^{95} in Food, Science.

(5) S. E. Zobrisky, H. D. Naumann, A. J. Dyer, and E. C. Anderson, The Relationship between the Potassium Isotope K^{40} and Meatiness of Live Hogs, J. Animal Sci.

CHAPTER 4

ORGANIC CHEMISTRY SECTION

Carbon^{14} in Lemongrass Oil (F. N. Hayes, E. Hansbury, and D. L. Williams)

Introduction

The program on contemporary C^{14} began as a test on the ultimate stability, sensitivity, and precision of the present state of the art of liquid scintillation counting and took on added significance from large-scale nuclear weapons testing because of the hazard of C^{14} to man and the useful data of a meteorological and geochemical nature which might result. Lemongrass oil is a natural terpene mixture which, by chemical conversion to p-cymene, can be incorporated into a 90-ml liquid scintillation counting volume and contribute 70 g of carbon. Lemongrass is commercially cultivated between the latitudes of 30°S and 25°N and is fast growing and frequently harvested.

Results and Discussion

Twenty-eight samples harvested between August 1955 and June 1959 have been counted and the results, corrected for fractionation, are shown in Fig. 1. The activity increases are grossly linear with a sharp break upward (t_b) at about June 1958. The two hemispheres have different slopes and intercepts. At t_o (January 1954), the time at the start of the activity rise above the prebomb level, the southern hemisphere extrapolates to be 2 per cent richer in C^{14}. The time of cross-over for the two hemispheres is October 1956. As of June 1959, the lemongrass data show the hemisphere increases to be 26.8 per cent in the north and 19.7 per cent in the south. As of this time, the average tropospheric content was 7.3×10^{27} atoms of bomb C^{14}.

An extremely simple three compartment model with exponential mechanism of transfer allowed from compartment one to two and from two to three has been applied to the experimental northern hemisphere lemongrass data. The compartments are stratosphere, into which the C^{14} is originally injected; troposphere, in which we are making activity measurements; and ocean, into which most of the activity will eventually go. Two mean residence times, τ_1, for stratosphere, and τ_2, for troposphere, are in the equation for the model, and the calculation has used 7 years for τ_2 and 1.5, 5, and 10 years

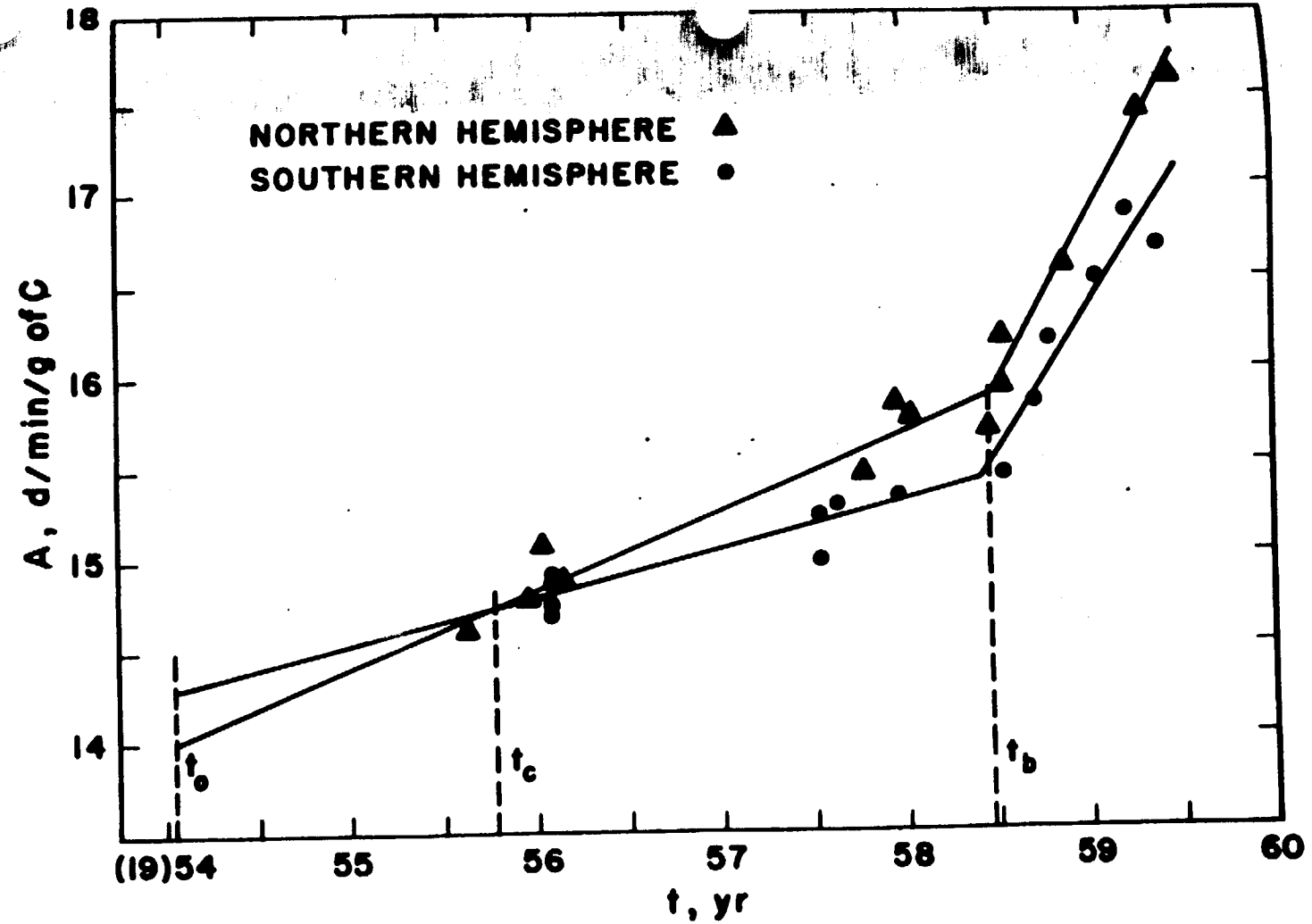


Fig. 1. Activities of C^{14} in lemongrass oil at various harvest times since large-scale nuclear weapons testing began.

for τ_1. Predictions into the near future with this model show peak activities and times varying with τ_1. The values are: for τ_1 = 10 years, the maximum increase in activity will be 56 per cent in September 1965; for τ_1 = 5 years, 44 per cent in May 1963; and for τ_1 = 1.5 years, 31 per cent in November 1960. This model also gives numbers for total stratospheric injections for the three values of τ_1. These are: for τ_1 = 10 years, injection = 5.9×10^{28} atoms of C^{14}; τ_1 = 5 years, 3.4×10^{28}; and τ_1 = 1.5 years, 1.6×10^{28}.

This program will continue at the rate of about 12 samples per year to provide data on the continuing rise, the eventual peak, and the decrease in C^{14} activity in the biosphere as the ocean proceeds to absorb most of this man-made isotope.

Carbon^{14} in Turpentine (V. N. Kerr, F. N. Hayes, and E. Hansbury, in cooperation with N. T. Mirov of the Department of Agriculture, Forestry Service, Berkeley, California)

Insufficient data have been collected to date for an accurate picture of C^{14} in turpentine. Only 22 samples have been measured. Four samples from the northern hemisphere, which were collected in 1954, gave an average of 13.98 $\pm$ 0.03 d/min/g of carbon. Nine samples collected in the southeastern part of the United States during the month of July 1955 averaged 14.21 $\pm$ 0.03 d/min/g of carbon.

A plot of the data through late 1958 shows that turpentines have risen in activity at about the same rate as lemongrass oil; however, there was a delay approaching this rate leading to an eventual time scale displacement (integrating time) for turpentines on the order of one year. Later samples should allow a more accurate assessment of this value.

Carbon^{14} in Citrus Fruit Oils (V. N. Kerr, F. N. Hayes, a
E. Hansbury)

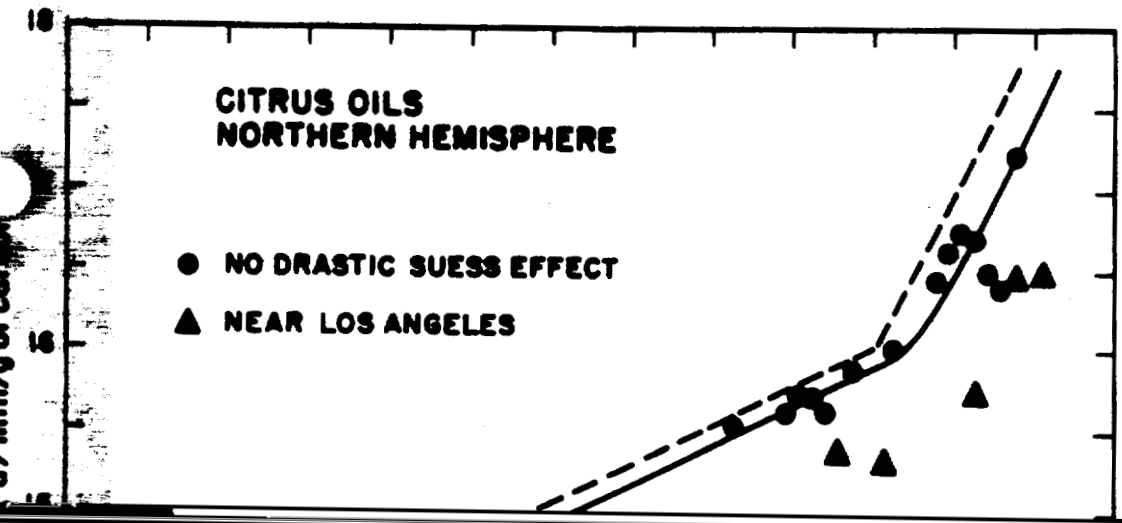
A program for measurement of C^{14} activity in citrus oils was originally instituted to supplement measurements taken from lemongrass oils and reported in the previous papers. The data provided by the larger number of citrus oils plus their wider geographical range serve as a basis for a pattern of C^{14} distribution and at the same time point up anomalies in the pattern.

The data from the 63 citrus oil samples measured to date cover the period from late 1955 through the first hal of 1959. The rate of increase of activity in these oils is parallel to that of the lemongrass samples. There is a displacement of the citrus oil values in time with regard to the lemongrass values. This is most probably due to t relatively longer growing period of the citrus fruits, wh an averaging of activities is obtained by laying down of materials of different activity during the growth period for the fruit. This apparent time difference between the lemongrass samples and the citrus oils is on the order of three months.

One obvious incongruity was provided by samples obtained from orange groves near Los Angeles. The much lower values obtained from these samples are interpreted

as a localized "Suess Effect." The difference between these oils and those produced in a more rural atmosphere averages about 5 per cent.

Figure 1 shows the rate of rise of C^{14} activity for the northern hemisphere, as shown in both lemongrass (dashed line) and citrus oils (solid line). The points falling below the line for normal oils are those from fruit grown near Los Angeles.



Carbon^{14} in Old Essential Oils (F. N. Hayes, V. N. Kerr, E. Hansbury, and D. L. Williams)

A program to measure contemporary increases in C^{14} activity in the biosphere must look backward into the near past to establish the level or trend in activity before the spectacular change being wrought by large-scale nuclear weapon testing can be evaluated. Old essential oils have been measured for C^{14} activity to establish their levels prior to 1954.

Six oils dating from 1940 to 1953 (one lemongrass, three citrus, and two gum turpentine) have been counted, and the resulting age- and fractionation-corrected C^{14} activities have been correlated with a 0.05 per cent per year Suess effect decrease in activity to give the equation

$$A = 14.38 - 0.007t$$

where A is d/min/g of carbon, and t is years after 1900. This equation and the equation of the best fit to the northern hemisphere lemongrass data from August 1955 to January 1958 give a simultaneous solution for t = 54.3 (April 1954) and A = 14.00.

The counting of camphor samples harvested during the last 15 years gives an essentially constant activity:

$$A = 14.37 \pm 0.04$$

Camphor is a very poor contemporary atmospheric sampler, since the tree from which it is derived is many decades old at time of harvest and has been storing camphor throughout its life. Some turpentines from pine stumps whose cutting time is established to have occurred many decades ago are being collected. When counted, their data will represent C^{14} activities before or in the early days of the era of large-scale burning of fossil fuels.

Contemporary C^{14} in the Zoosphere (V. N. Kerr. E. Hansbury, and F. N. Hayes)

This is a new program and as such very little beyond the development of technique has transpired. Since there are no plants of marine origin which yield products capable of being used for C^{14} assay with the same facility as the essential oils, marine animal products have been investigated as a source of samples. Spermacetti wax from whales seems to be the best choice for assay at present.

In addition to the marine animal data, there will be some from land animals and humans. Two techniques are being developed: one is the assay of cholesterol dissolved in a standard counting solution, and the other involves the synthesis of a "live" solvent from "dead" benzene and isopropyl iodide obtained from glycerol (a component of fat).

Liquid Scintillation Solutes (F. N. Hayes, E. Hansbury, V. N. Kerr, and D. G. Ott)

The scintillator testing program, which to date has evaluated over 500 compounds as scintillation solutes, is continuing in cooperation with G. Daub of the University of New Mexico and his graduate students who carry out scintillation absorption and fluorescence studies with the facilities in H-4. A study of phenanthrene derivatives has been completed in the dissertation of S. P. Birkeland (1), who found seven new solutes more efficient than PPO (our standard), two of which matched the performance of our best solute PBD. One of these new compounds, 5,7-dihydro-3,9-diphenyldibenz [c,e] oxepin (D-52), is finding application as the short wave length emitter in a two-scintillator detector under development by F. Reines at the Case Institute of Technology. Special naphthalene derivatives and stilbene analogs are now being studied.

There has been no solute in all the testing program with a maximum relative pulse height (RPH) greater than 1.28. We have received a private communication in manuscipt form from G. Herrmann, Johannes Gutenberg Universität, Mainz, Germany, in which he reports two new solutes with maximum RPH >1.45. These were evaluated in a system and with a standard supposedly identical to ours. We received samples

of these two solutes from him and found both to have maximum RPH = 1.18. This discrepancy is being further investigated both here and in the German laboratory.

Shimanskaya, et al (2) have reported that a new solute, 2-(4-biphenylyl)-5-(1-naphthyl)-1,3,4-oxadiazole, is 20 percent better than our best solute (thus its RPH should be about 1.54). We have synthesized the Russian compound and find that its maximum RPH is 1.13.

REFERENCES

(1) S. P. Birkeland, University of New Mexico Report, "Liquid Scintillators: Some Aryl Substituted Phenanthrenes and Dihydrophenanthrenes, and Related p-Terphenyls and p-Quaterphenyls" (1959).

(2) N. P. Shimanskaya, A. P. Kilimov, and A. P. Grekov, Optika i Spektroskopiya 6, 194 (1959).

New Liquid Scintillators for Large Volume Applications

(V. N. Kerr, F. N. Hayes, D. G. Ott, and R. L. Schuch)

The very large volume (~400 gallons) of Humco II (see Chapter 3) made it necessary to look for new scintillator solvents with the economy and transparency of toluene (solvent in Humco I), along with the high flash point of triethylbenzene (the solvent in Genco (1)). Solvent transmissibility and relative pulse heights (RPH) of several solvent, solute, scintillator combinations were measured using 12-liter volumes of scintillator solution. Table 1 shows some of the results and additional information concerning a few new solvents which indicate their possible usefulness as large volume scintillator fillings.

By the time of the next report, it is hoped not only to have chosen a worthy scintillator for Humco II, but also to have gained considerably more understanding as to the relative importance of various scintillation parameters in affecting the performance of a large volume scintillation detector.

REFERENCE

(1) E. C. Anderson, F. N. Hayes, and R. D. Hiebert, Nucleonics 16(8), 106 (1958).

TABLE 1. SOLVENT CHARACTERISTICS AND RELATIVE PULSE HEIGHTS OF 12-LITER VOLUMES OF VARIOUS SOLVENT, SOLUTE, SCINTILLATOR COMBINATIONS

Solvent	RPH with 3 g/l PPO	A_s (440 mμ) (meters)	Flash Point (°C)	Solutes and Concentration (g/l)	RPH
Toluene (AR)[a]	1.00	100	7	Terphenyl (4) POPOP (0.05)	1.00
Monoisopropyl-biphenyl[b]	0.92	0.78	139	Terphenyl (4) α-NOPON (0.05)	1.11
Saf-T-Sol 105[c]	0.88	10.7	93	---	----
ScinSol I[d]	0.74	2.5	130	PPO (5) α-NOPON (0.03)	0.70
TS-28[e]	0.72	100	50	---	----

(a) Mallinckrodt Chemical Company.

(b) Treated by passage through a column of activated alumina. Monsanto Chemical Company.

(c) Distilled from sodium. Crowley Tar Products Company.

(d) Borden Chemical Company.

(e) Distilled from sodium. Shell Oil Company.

Liquid Scintillation Counting of Tritium (D. G. Ott, F. N. Hayes, and T. T. Trujillo)

Introduction

The liquid scintillation counting system which has been in routine use for several years, although quite satisfactory for most tritium assays, has been known for some time to be capable of considerable improvement. In the first place, it is aesthetically desirable to be using the best possible system available (within certain bounds of convenience and economics) and secondly, to have a system which is capable of assaying tritium water samples whose activities are very low (a few times background). Since these properties have not been present, investigations were conducted to determine the formula and properties of an improved solution whose principal use would be for assaying one-ml water samples.

Results and Discussion

Concentration of the components of the dioxane system were varied and the following formula was chosen: naphthalene, 125 g/l; PPO (recrystallized), 7.5 g/l; POPOP, 0.375 g/l; and dioxane.* Using 15 ml of solution, efficiencies for counting tritium were about 10.8 per cent with 0.1 ml water; 9 per cent with 1 ml water; and 4.5 per cent with 3 ml water. This

*Eastman Kodak Company No. 2144.

represented an improvement of about 50 per cent over the "old" solution. The Kimble 10-dram Opticlear Vial originally used has been replaced by the 20-ml screw-cap Wheaton Liquid Scintillation Spectrometer Vial. Background is about 35 c/min using 15 ml of scintillator solution.

Dioxane systems have been observed to exhibit the phenomenon of chemiluminescence resulting in extremely high background of very short half-life. This effect is to be studied in detail, but in practice it has been found to occur if the sample is basic and it can be eliminated by lowering the pH with a few microliters of concentrated hydrochloric acid.

With certain low activity samples containing quenching impurities, it is not possible to prepare a blank of identical characteristics for background determination. Following from the previous demonstration for C^{14} that background can be related to counting efficiency, a similar relationship will be sought for tritium water counting.

Further studies to be carried out very soon include development of a more satisfactory internal standard technique, the problem of increased efficiency at low counting rates; and determination and improvement (if necessary) of the reliability, stability, and reproducibility of the entire counting system, particularly for low activity samples. Limitation on the amount of tritium activity that can be administered to

people in the course of diagnostic and physiological study necessitates the development of more precise and rapid techniques of low level tritium counting, if the potential value of this isotope in such studies is to be realized.

Chemical Systems for Liquid Scintillation Dosimetry (D. L. Williams and F. N. Hayes)

Introduction

Liquid scintillator systems which are intended for use in making radiation rate measurements must be designed with specific types of radiation in mind. For example, dependent upon the source, the radiation may be: (a) gamma rays only; (b) fast neutrons only; or (c) a mixed field of gamma rays plus fast and thermal neutrons.

Any good hydrocarbon liquid scintillator can be calibrated to measure dose rates in cases (a) and (b) above, but the ideal system with two scintillators, one of which is specific for gamma rays and the other specific for fast neutrons, does not appear attainable. However, a less ideal system for measuring gamma and fast neutron dose rates in a mixed radiation field is possible. Since scintillator response to gamma rays is dependent upon electrons and response to fast neutrons is dependent upon hydrogen atoms, a two-component system is possible in which one scintillator (with no hydrogen atoms) responds only to gamma rays and the other responds to both gammas and fast neutrons. Thus, with the proper calibrations, the fast neutron rate is obtained as a calculated difference value.

Results and Discussion

Of the organic compounds which do not contain hydrogen, perfluoro- compounds such as perfluorohexane, hexafluorobenzene, and perfluorotoluene looked most promising as liquid scintillator solvents. Perfluorohexane was prepared but was an extremely poor solvent for scintillator solutes. Perfluorotoluene has been obtained in small yield only as a by-product. Consequently, hexafluorobenzene was prepared essentially according to the procedure of Hellmann, et al (1), and purified by the gas chromatographic technique.

Solutions of various solutes in hexafluorobenzene gave elative currents (2) ranging up to 25 per cent of the 2,5-diphenyloxazole-toluene standard. Based upon light output and solubility, diphenyloxazole was the solute of choice but its solution in C_6F_6 proved to be more subject to radiation damage at high dose rates than were solutions of 9,10-diphenylanthracene. Oxygen-free solutions of the latter solute in hexafluorobenzene (2.25 g/l) were the least affected of those investigated, and oxygen removal by saturating the solutions with argon increased scintillation efficiency by 64 per cent.

Based upon relative current and oxygen quenching considerations, a solution of 9,10-diphenylanthracene (3 g/l) in p-xylene was chosen for the neutron sensitive medium. Purging of this solution with argon raised the response to Co^{60}

gamma rays by 67 per cent. As used in photodetectors for measuring radiation rates, the ratio of response of the p-xylene system to gamma rays and to fast neutrons was 4.6 to 1. With the accuracy of these measurements considered to be ± 5 per cent, the maximum ratio of gamma to fast neutron rates at which a significant neutron measurement may be made is about 4.3 to 1.

It is considered highly desirable to increase the response of the hydrocarbon system to fast neutrons. Liquid scintillators are being prepared currently which include elements such as gadolinium and europium, with large fast neutron cross sections. These elements are made soluble via their 2-ethylhexanoate salts.

REFERENCES

(1) M. Hellmann, E. Peters, W. J. Pummer, and L. A. Wall, J. Am. Chem. Soc. 79, 5654 (1957).

(2) B. S. Rogers, P. C. Sanders, R. L. Schuch, D. L. Willi and F. N. Hayes, Los Alamos Scientific Laboratory Repo LA-1639 (1953).

Photodetectors for Liquid Scintillation Dosimetry in the Field (D. L. Williams, F. N. Hayes, and R. L. Schuch)

Dose rate devices are needed which are capable of measuring the dose rate of each component of a mixed radiation field in the vicinity of reactors and other large sources. A specific example of such a need is the recent measurement of radiation dose rate from Kiwi-A, the first prototype of a nuclear power unit for rocket propulsion. These devices should be dose rate and energy independent over the applicable energy region. Other desirable features are small size, mobility, and a minimum requirement for associated electronic equipment.

To go with the system of "paired" liquid scintillator detectors (described in a previous report), which had already been developed to meet these requirements, it was necessary to find suitable photodetectors in order to produce practical liquid scintillation dosimeters. A detector for high dose rates was constructed from a cylindrical steel chamber with 20-ml volume, one end of which was a 1-1/8 in. diameter selenium-barrier photovoltaic cell (Fig. 1). The inner surface of the steel chamber was coated with an epoxy resin, containing anatase TiO_2. The detector used, as described in Los Alamos Scientific Laboratory Report LA-2375 (1), gave a useful linear response from 6 to 1 x 10^5 rad/min of gamma

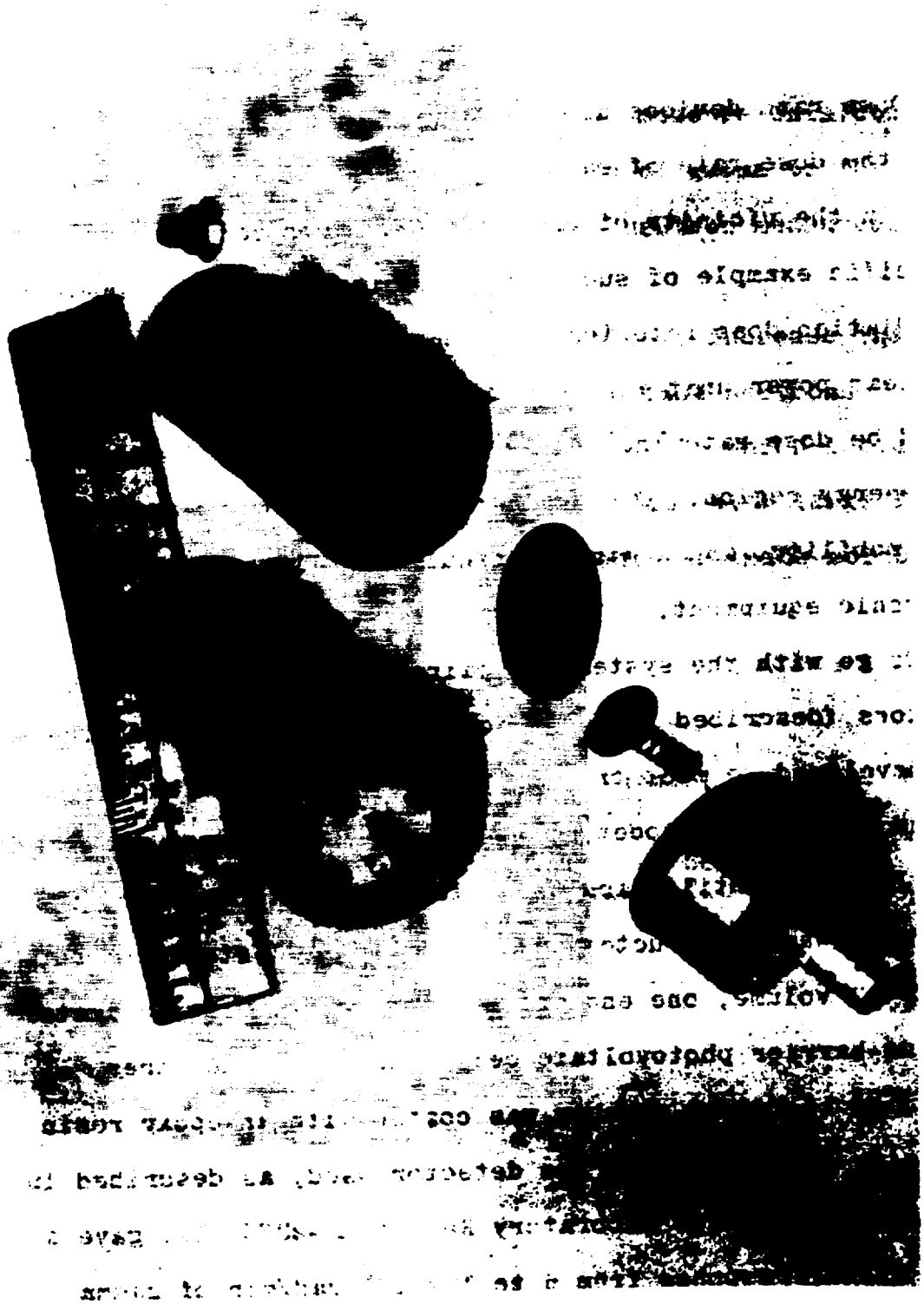


Fig. 1. Photovoltaic cell and 20-ml chamber. In final assembly, the cell is cemented into chamber to make a liquid-tight seal.

excitation. Response of the unit (in a mixed radiation field) filled with diphenyloxazole-p-xylene scintillator is shown in Fig. 2, and its response with a diphenyloxazole-hexafluorobenzene filling is shown in Fig. 3. A detector for low dose rates was like the above except that the cathode end of a DuMont 6467 photomultiplier was made to replace the photovoltaic cell. This detector (1) was linear from 10^{-5} to 10^{2} rad/min.

The systems described are linear over an extremely wide range and are capable of transmitting signal over long distances. Simultaneous gamma and neutron rate measurements can be made using pairs of detectors filled with the appropriate liquid scintillators. The basic limitations are: (a) the necessity for preparation of the hexafluorobenzene solvent; (b) the deterioration of all organic materials, as well as photovoltaic cells over a period of time in high radiation fluxes; and (c) neutron energy dependence of the neutron plus gamma ray system. In the latter case, the problem from the practical point of view may be overcome through proper calibration. Work on these systems with different scintillator fillings and known radiation will continue.

REFERENCE

(1) D. L. Williams, F. N. Hayes, R. L. Schuch, R. L. Crawford, and R. D. Hiebert, Los Alamos Scientific Laboratory Report LA-2375 (1959).

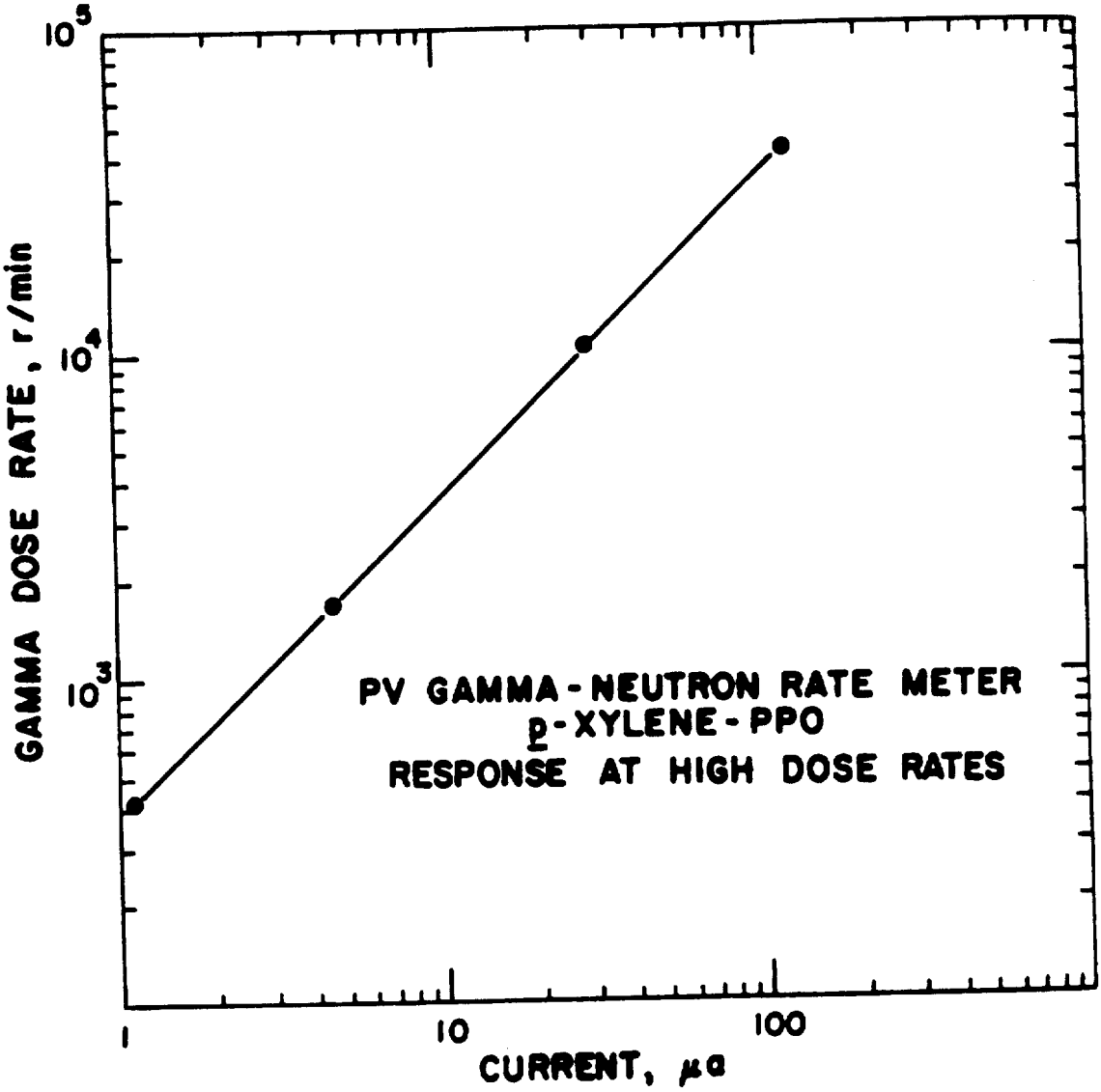


Fig. 2. Response of photovoltaic cell detector to gamma rays plus fast neutrons in a mixed radiation field.

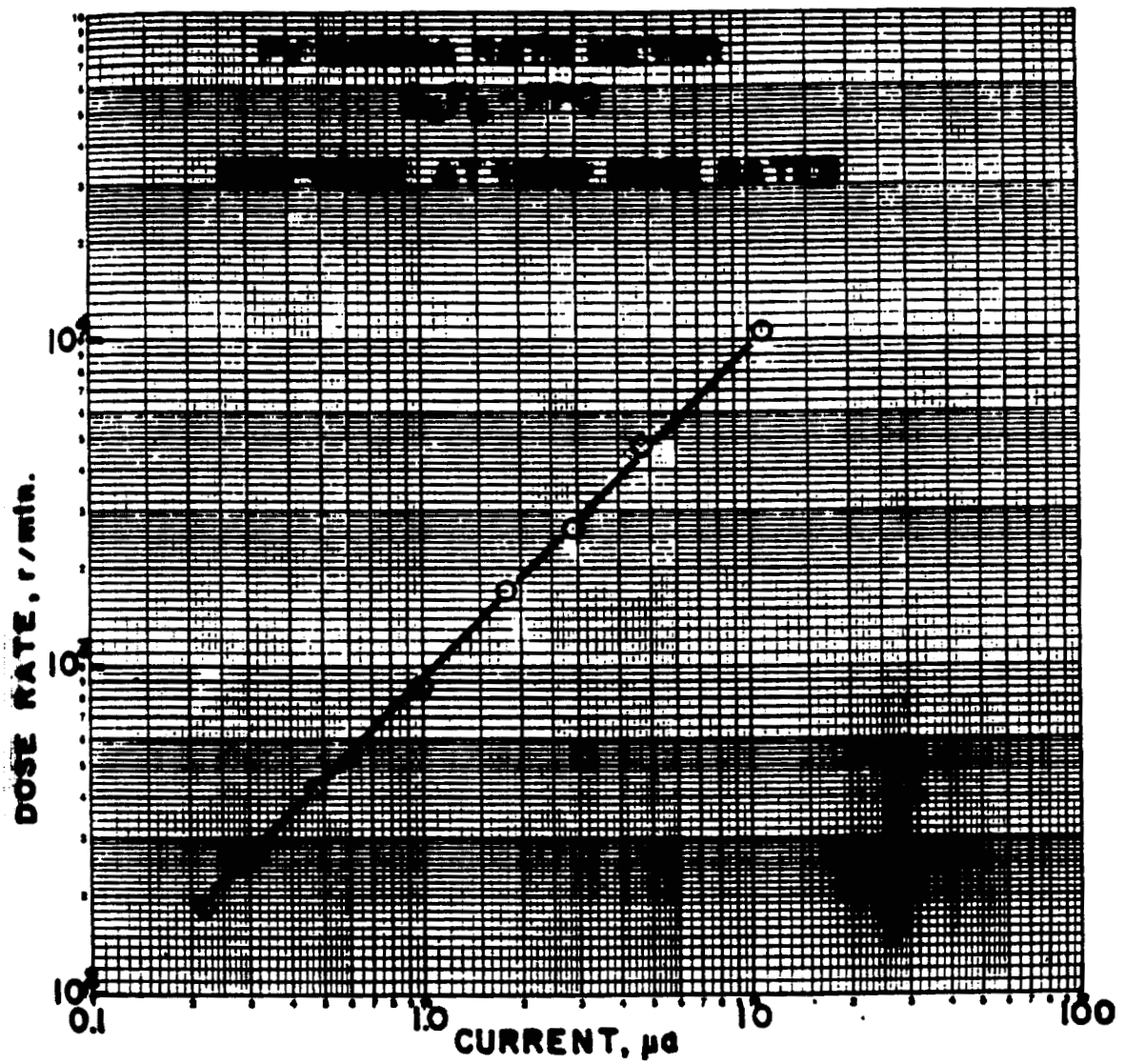


Fig. 3. Response of photovoltaic cell detector to gamma rays only in a mixed radiation field.

Construction of a Preparative-Scale Gas Chromatographic Column (D. L. Williams)

A mobile preparative-scale gas chromatographic unit has been assembled (Fig. 1), which is capable of handling gram quantities of material. The sensing device is a thermal conductivity cell which actuates a strip chart recorder. By means of bridge current and sensitivity controls, a wide range of detector sensitivity is available. Components which amount to as little as 0.06 per cent of a 1-gram sample are detectable. The 2-in. x 10-ft. column in this unit is quickly and easily interchangeable with others, thus offering ready access to a variety of column packing materials.

Fig. 1. Preparative-scale gas chromatographic assembly.

Assembly of Spectrophotometric Facility (D. G. Ott)

All spectrophotometric instruments of the Group have been centralized in a single instruments laboratory, which allows more efficient and convenient use of the equipment for obtaining physical properties and for qualitative and quantitative analyses. Virtually all projects in the Organic Chemistry Section (as well as some from other sections) have made increasing use of the facility. The instruments available are a Beckman DK-1 Recording Spectrophotometer for the ultraviolet, visible, and near-infrared (210 to 2850 mμ); a Baird Recording Infrared Spectrophotometer for the infrared (2 to 16 μ); and a recent addition, an Aminco-Bowman Spectrophotofluorometer, modified for use with a Varian recorder, for fluorescence measurements in the ultraviolet and visible regions. A set of Sadtler Standard Spectra for the infrared and near-infrared is available.

Labeling of Biologically Important Compounds with Radioactive Isotopes (A. Murray)

The programs of the Biochemistry, Radiobiology, and Radiopathology Sections have been supported by the synthesis of the following labeled compounds, the figures in parentheses being specific activity of the radiochemically pure compound (in mc/g) on a carrier-free basis:

(a) C^{14}-Isoniazid (14.05).

(b) H^3-N,N',N''-Triethylenethiophosphoramide (2.15, 5.66, and 6.92).

(c) H^3-Cholesterol (39.3).

(d) H^3-Mevalonic acid as N,N'-dibenzylethylenediamine salt (14.3).

(e) H^3-1-[p-(β-diethylaminoethoxy)-phenyl]-1-(p-tolyl)-2-(p-chlorophenyl)ethanol (35.3 and 375).

(f) H^3-Pyridoxine hydrochloride (1.56 and 1,310).

(g) H^3-Thymidine (395, 516, and 2,100).

(h) H^3-Thymine (19,250).

(i) H^3-Deoxycytidine (8,640).

(j) H^3-1-Acetyl-2-isonicotinoyl hydrazine (5.63).

(k) Dihydrolanosterol-25,26-H_2^3 (0.086).

With F. Domer* we prepared the C^{14}-Hemicholinium compound (a cholinesterase inhibitor): [4,4'-Biphenylenebis(2-oxoethethylene)] bis [(2-hydroxyethyl)dimethylammonium-C_1^{14} bromide].

*From the Department of Pharmacology, Tulane University School of Medicine, New Orleans, Louisiana.

In addition, the following labeled compounds are in the process of being synthesized:

(a) 2,4,6-Trinitrotoluene-1-C^{14}.

(b) Dihydrolanosterol-25,26-H_2^3 (in high activity).

(c) H^3-Lanosterol.

(d) H^3-Linoleic Acid.

Analytical and Separation Methods of Special Use in Multi-site Destructive Labeling Methods (A. Murray)

Application of paper chromatography (employing 14 x 22 in. sheets of Whatman No. 3 MM, No. 17, and seed test papers) has been developed for separation and recovery of C^{14}- or H^3-labeled components of a mixture of organic compounds up to the level of 75 mg to 2 g per sheet. The method has proved to be particularly effective in purification of tritium-labeled products resulting from the gas-exposure technique, as well as those of extremely high activity formed by catalytic exchange in the presence of HTO (1300 c/4 ml). A simple exposure to X-ray film, as the detecting system, replaces the need for counting and analyzing the hundreds of aliquots of eluate collected by the usual preparative column chromatography methods. Another detector applicable to ultraviolet-absorbing materials is the ultraviolet scanner camera (1). A simple fluorescent intensification screen has been developed to extend its usefulness.

The large paper method has been applied to isolation from human urine of C^{14}-metabolites of the drug isoniazid in amounts sufficient to permit identification through their physical properties.

A study of the transformation brought about in aqueous thymidine solution under conditions of radiolysis indicates

that the products are not those of simple hydrolysis to thymine and deoxyribose. It is hoped that these separative methods will prove of value in establishing their identity.

REFERENCE

(1) N. A. Drake, W. J. Haines, R. E. Knouff, and E. D. Nielson, Anal. Chem. 28, 2036 (1956).

ORGANIC CHEMISTRY SECTION PUBLICATIONS

(1) M. D. Barnett, G. H. Daub, F. N. Hayes, and D. G. Ott, Liquid Scintillators. VI. 2-Aryl- and 2,7-Diaryl-fluorenes, J. Am. Chem. Soc. 81, 4583 (1959).

(2) H. R. Baxman, D. D. Jackson, D. L. Williams, and R. J. Bard, Preparation of Pitch-Soluble Uranyl-Organic Compounds. Los Alamos Scientific Laboratory Report LA-2320 (1959).

(3) V. N. Kerr, F. N. Hayes, D. G. Ott, and E. Hansbury, Liquid Scintillators. VII. 2,5-Diaryl Substituted Thiazoles as Liquid Scintillator Solutes, J. Org. Chem. 24, 1861 (1959).

(4) V. N. Kerr, F. N. Hayes, D. G. Ott, R. Lier, and E. Hansbury, Liquid Scintillators. VIII. The Effect of the Dialkylamino Group, J. Org. Chem. 24, 1864 (1959).

(5) D. G. Ott, C. R. Richmond, T. T. Trujillo, and H. Foreman, Cab-O-Sil Suspensions for Liquid Scintillation Counting, Nucleonics 17(9), 106 (1959).

(6) D. G. Ott, The (5-Phenyl-2-oxazolyl)pyridines as Fluorescent pH Indicators, An Application to Chemical Radiation Dosimetry. In: Chem. Ab. 53, 11000 (1959).

MANUSCRIPTS SUBMITTED AND ACCEPTED

(1) M. D. Barnett, G. H. Daub, F. N. Hayes, and D. G. Ott, Liquid Scintillators. XI. 2-(2-Fluorenyl)-5-aryl-substituted oxazoles and 2-(2-fluorenyl)-5-phenyl-1,3,4-oxadiazole, J. Am. Chem. Soc.

(2) F. R. Domer and F. N. Hayes, A Correlation of Counting Efficiency and Background, Nucleonics.

(3) F. N. Hayes, E. Hansbury, and V. N. Kerr, Contemporary Carbon^{14}: The p-Cymene Method, Anal. Chem.

(4) F. N. Hayes, E. Hansbury, V. N. Kerr, and D. L. Williams Contemporary Carbon^{14} in Lemongrass Oil, Zeit. Physik.

(5) V. N. Kerr, D. G. Ott, and F. N. Hayes, Quaternary Salt Formation of Substituted Oxazoles and Thiazoles, J. Am. Chem. Soc.

(6) D. G. Ott, Advances in Liquid Scintillation Counting, to be published in TID-7578. Proceedings of the Fourth Conference on Radioactive Isotopes in Agriculture, Oklahoma State University (April 2, 1959), General Paper No. 8.

(7) D. G. Ott, V. N. Kerr, F. N. Hayes, and E. Hansbury, Liquid Scintillators. XII. Absorption and Fluorescence Spectra of 2,5-Diaryl-1,3,4-oxadiazoles, J. Org. Chem.

(8) D. L. Williams, F. N. Hayes, R. L. Schuch, R. L. Crawfor and R. D. Hiebert, to be published as Los Alamos Scienti Laboratory Report LA-2375.

CHAPTER 5

RADIOBIOLOGY SECTION

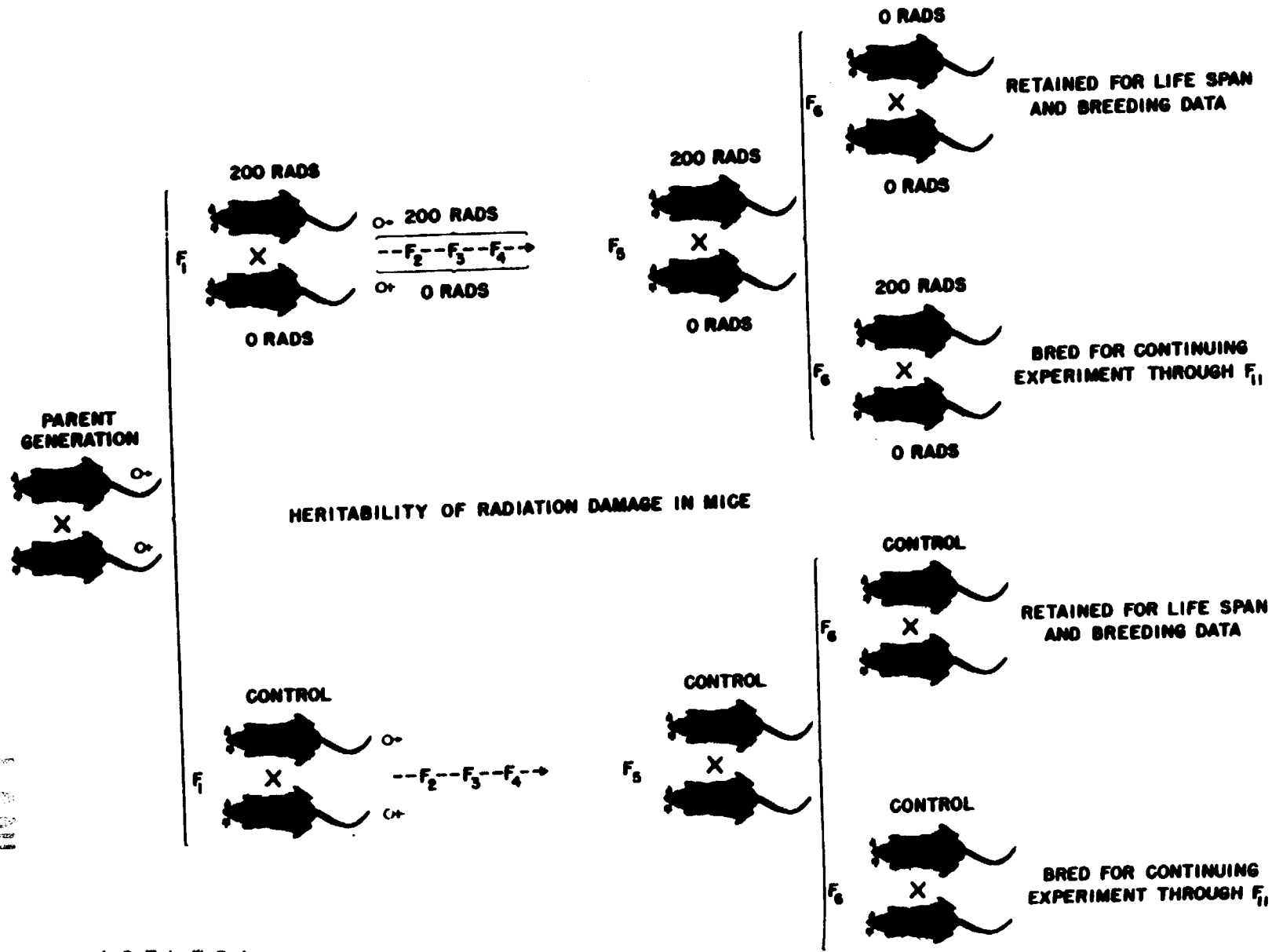
Heritability of Radiation Damage in Mice (J. F. Spalding and V. G. Strang

Introduction

There is increasing evidence that many mutations may have slight dominant deleterious effects, which might culminate in a greater population damage than the lethal effects of homozygous mutations (1). The study described here was designed to observe the heritability of radiation damage in mice in terms of reproductive performance and life span.

Methods and Results

The population of mice used in this study originated from a single pair of mice, and were treated as shown in Fig. 1. The X-ray treated mice were exposed acutely at 4 weeks of age. At the present time only the reproductive performance of the 6th generation has been completed. Since these data


PARENT GENERATION
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X
♂
F_1
200 RADS
X
0 RADS
♂ 200 RADS
--F_2--F_3--F_4→
♀ 0 RADS
F_5
200 RADS
X
0 RADS
F_6
0 RADS
X
0 RADS
RETAINED FOR LIFE SPAN AND BREEDING DATA
F_6
200 RADS
X
0 RADS
BRED FOR CONTINUING EXPERIMENT THROUGH F_{11}
HERITABILITY OF RADIATION DAMAGE IN MICE
F_1
CONTROL
X
♂
♀
--F_2--F_3--F_4→
F_5
CONTROL
X
F_6
CONTROL
X
RETAINED FOR LIFE SPAN AND BREEDING DATA
F_6
CONTROL
X
BRED FOR CONTINUING EXPERIMENT THROUGH F_{11}

are preliminary, no attempt has been made to analyze and refine them. The mean and standard errors of each observation, together with a comparison of the control and experimental groups, are shown in Table 1. The preliminary results of the litter data strongly suggest that sublethal (undesirable) hereditary determiners are produced by acutely administered X rays of the magnitude used and that these undesirable characteristics are passed on to future generations in the manner one would expect from random segregation. The extent to which these characteristics are additive may be determined when the litter data from the 12th generation are completed.

Reference

(1) W. L. Russell, L. B. Russell, and E. F. Oakberg, "Radiation Genetics of Mammals." In: Radiation Biology and Medicine, W. D. Claus (ed.), Addison-Wesley, Reading, Mass. (1958).

TABLE 1. Observations on Sixth Generation and Their Litters

Observations	Control	Experimental	% of Control
Age at first conception (days)	60 ± 2*	63 ± 3*	95
Age at last conception (days)	238 ± 16	222 ± 20	93
Reproductive life (days)	178 ± 16	158 ± 20	89
Average number of conceptions per female	5.2 ± 0.4	4.4 ± 0.6	85
Average number of mice born per female	32.7 ± 2.5	22.8 ± 3.8	70
Average number of mice weaned per female	27.6 ± 2.5	19.7 ± 3.4	71
Average litter size born	6.6 ± 0.26	4.6 ± 0.54	70
Average litter size weaned	6.1 ± 0.32	4.5 ± 0.56	74
Average total weight of mice weaned per female	314 ± 29	180 ± 32	57
Average weaning weight	11.4 ± 0.22	10.4 ± 0.34	91
Sex ratio of litters (female to male)	0.99	0.98	No difference

*Standard error.

A Life Span Study of First and Second Generation Offspring from Irradiated Spermatids and/or Sperm Cells, and from Irradiated Type A Spermatogonia of Sires and Grandsires

(J. F. Spalding and V. G. Strang)

Introduction

There has been some evidence to indicate that genetic factors which determine the life span of a species may be affected adversely in the first generation offspring of male mice exposed to fission neutrons (1). Evidence has also been introduced which indicates that mutation rates may differ with spermatogonial stage (2). The purpose of this experiment is to study the relative effects of fission neutron and Co^{60} gamma irradiation of the male parent on the life span and viability of his offspring, as produced from an early and a late breeding. In the early breeding, the offspring resulted from sperm that were in the late stage of development at time of irradiation, and in the late breeding from sperm that were in the very early stage of development.

Methods and Results

Two hundred and twenty RF male mice 8 weeks of age were randomly divided into 11 groups of 20 mice each and treated as shown in Fig. 1. Groups 1 through 5 were given 32, 67, 102, 134, and 177 rads of fission neutrons, respectively.

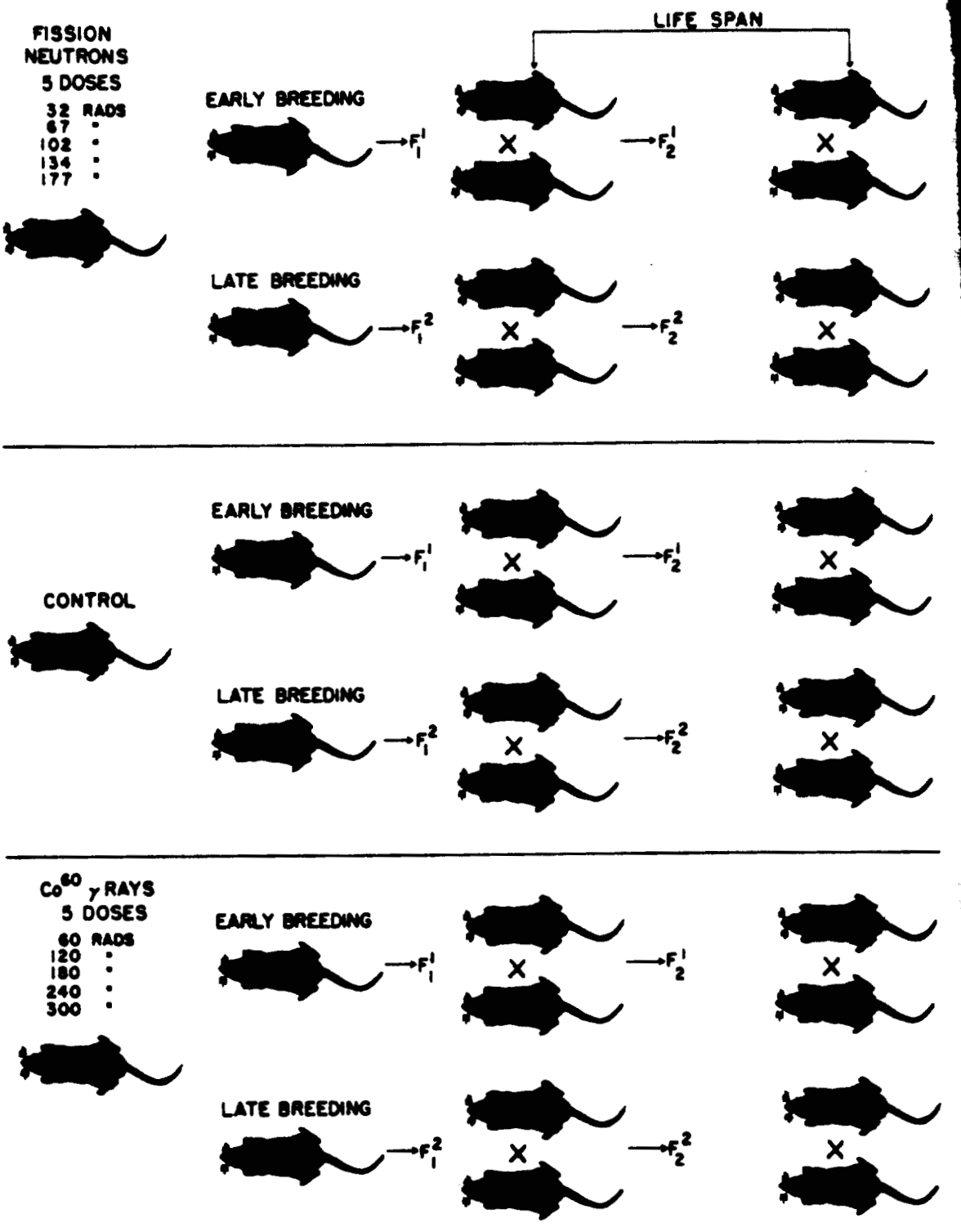

Fig. 1. Life span study of the F_1 and F_2 of male mice exposed to fission neutrons or Co^{60} gamma rays.

Groups 6 through 10 were given 60, 120, 180, 240, and 300 rads of Co^{60} gamma rays, respectively, and group 11 was retained as an unexposed control group.

Following exposure, all male mice from exposed and control groups were placed in breeding cages with a randomized population of RF female mice 10 to 12 weeks of age with a cage ratio of 1 male to 4 females. Eighteen days after the mating date, the males were removed. The longevity of offspring from these matings is being studied. Offspring of irradiated sires in part (1) were sib-mated, and the longevity of their offspring is being studied.

Sixteen weeks following exposure, all irradiated and control males were placed in breeding cages with a randomized population of RF female mice 10 to 12 weeks of age with a cage ratio of 1 male to 6 females. The longevity of offspring from these matings is under study. Offspring of irradiated sires in part (3) were sib-mated, and the longevity of their litters is being studied.

This program was started in September 1958 and will terminate about September 1961.

References

(1) W. L. Russell, Proc. Nat. Acad. Sci. 43(4), 324 (1957).

(2) W. L. Russell, J. W. Bangham, and J. S. Gower, Proc. 10th Intern. Congress Genet., Montreal 245 (1958).

Comparison of Natural and Radiation Aging Mechanisms. -- Response of Irradiated and Nonirradiated Mice to Cold Stress (T. T. Trujillo and J. F. Spalding)

Introduction

Considerable information is available suggesting that young and old animals show significant differences in their ability to withstand stress. The response of two age groups appears to vary with life span interval of the species and degree of stress. Subnormal temperature is a nonspecific stress to which young mice appear to adjust better than do old ones, as indicated by the per cent of survivors. It was observed that at a temperature of 6 to 7°C for 14 days in individuals, 73 per cent of 6 month old mice survived, while only 17 per cent of 19 month old ones could withstand this stress.

It is well known also that a definite life shortening in mice results from single or multiple doses of whole body radiation. Variations in reduction of life span are observed with strain, sex, dose, and dose rate. A range in the reduction of life span in female mice of from 0.2 to 0.7 day per roentgen has been reported.

The aging process is poorly understood if at all; however, it seems important to compare the natural process with radiation aging under laboratory conditions. This investigat

currently in progress, was designed to observe differences in the two aging mechanisms in a single strain of irradiated and nonirradiated mice, when exposed to a nonspecific stress such as cold.

Methods and Results

Young female mice of the RF strain are being used in the investigation. The radiation exposures are gamma rays from a Co^{60} source. The dose rate and length of exposure can be varied to obtain desired dose of total body radiation and varied life shortening. Radiation exposures begin when animals are 3 to 4 months of age. At the termination of exposure, all groups are allowed a 90-day recovery period before subjected to stress. All deaths during this period would be considered acute deaths, and subsequent to this period as chronic deaths due to an aging process. With each group of irradiated mice exposed to the cold environment, a group of nonirradiated animals of the same chronological age is exposed and serve as controls. All cold stress periods are for 14 days at 6 to 7°C in a walk-in refrigerator. The animals are housed in individual compartments with food and water available ad libitum. Upon termination of stress, the mice are returned to the normal warm quarters for an additional 10-day survival observation period, after which the

experiment is terminated. To determine first the effect of natural aging on resistance to cold of the RF strain, 500 young female mice of the same age were randomized and groups of 100 each are being placed in the cold room every 4 months. The life span of the RF mouse is believed to be between 2 and 3 years.

The results to date are recorded in Tables 1 and 2. All groups were observed for deaths during and for 10 days after the stress period. Table 1 shows that 61 per cent of the irradiated mice died, compared to 45 per cent of the non-irradiated animals. Table 2 shows per cent deaths of 1 non-irradiated group, compared with 4 irradiated groups each receiving a different dose. Of the 500 young mice divided in groups of 100 each to be exposed to cold every 4 months to determine resistance to stress with age, only the first group (4 months old) has been stressed with 86 per cent surviving.

From the data presented, it is evident that there is a decline in the ability of irradiated mice to resist cold when stressed along with a nonirradiated group of the same chronological age. As shown in Table 2, the per cent deaths in the irradiated groups corresponded with the whole body dose received, with the exception of Group II which received the least radiation. There was no gross observation during the

TABLE 1. Effect of Cold Stress on the Survival of Young Irradiated and Non-irradiated Mice

Mice Stressed		Total[a] Dose (r)	Age at Start[b] of Stress (months)	Deaths during 14-day Stress (No.)	Deaths during 10-day Post Stress Period (No.)	Total Deaths (%)
Group	No.					
I	150	0	8	68	0	45
II	150	800	8	86	6	61

(a) At age of 12 weeks, Group II received 400 r in approximately 31 minutes and 2 subsequent exposures of 200 r each at 30-day intervals. All doses were delivered at a rate of 13 r/min from a Co^{60} source.

(b) Animals were allowed a 90-day recovery period between last exposure and start of cold stress.

TABLE 2. Effect of Cold Stress on the Survival of Young Irradiated and Non-irradiated Mice

Mice Stressed Group	No.	Dose (r)	Duration[a] of Irradiation (days)	Age at Start[b] of Stress (months)	Deaths during 14-day Stress (No.)	Deaths during 10-day Post Stress Period (No.)	Total Deaths (%)
I	96	0	0	7.5	13	0	14
II	96	494	7	7.5	57	1	60
III	96	967	14	7.5	29	2	32
IV	72	1481	21	7.5	26	2	39
V	43	1974	28	7.5	31	1	74

(a) Dose rate = 70.5 r/22-hour day from Co^{60} source. Exposure terminated at the same time for all groups.

(b) Animals were allowed a 90-day recovery period between termination of irradiation exposure and start of cold stress test.

stress or post stress period to indicate a reason for the high mortality in this group. To observe this phenomenon further, a similar experiment is currently under investigation. Four hundred RF female mice, 4 to 5 months old, in groups of 100 each, have received 512, 1522, 2598, and 3682 roentgens, respectively, at a dose rate of 50 r/day. These groups have not been exposed to cold as yet. From the data in Tables 1 and 2, it appears that the aging mechanisms of the natural and radiation processes are very similar; however, reduction of life span due to radiation for the RF female mouse, based on our dose rates, has not been observed.

Effect of Graded Acute Exposures of Gamma Rays and Fission Neutrons on Recovery from Subsequent Protracted Gamma Ray Exposures

(J. F. Spalding, V. G. Strang, and F. C. V. Worman)

Introduction

The measurement of "reparable" and "irreparable" components of injury from ionizing radiations in mammals is still the subject of much controversy (1,2). The study outlined here was designed to test the theory that the degree of resistance or survival time in a continuous field of low intensity gamma rays may be used as a measure of one component of irreparable damage from prior challenging doses of degraded fission neutrons and Co^{60} gamma rays.

Methods and Results

Five hundred and twenty-eight female mice were randomly divided into 12 groups and given total doses of gamma rays ranging from 240 to 1200 rads, or fission neutrons ranging from 92 to 451 rads (Table 1). To avoid possible acute radiation deaths, all doses were fractionated into 5 exposures delivered at 4-day intervals. After a repair period of 90 days, they were placed in a continuous field of gamma rays (50 rads per 24 hours), where they remained until death. The Mean Accumulated Dose (MAD) for each group was determined and correlated with the fractionated acute challenging dose.

TABLE 1. Challenging Doses of Gamma Rays and Fast Neutrons*

Gamma Rays			Fission Neutrons		
Group No.	No. of Mice	Total Dose (rads)	Group No.	No. of Mice	Total Dose (rads)
1	42	240	6	42	92
2	42	480	7	42	182
3	44	720	8	44	271
4	48	960	9	46	355
5	50	1200	10	48	451
[illegible]	40	Zero	12	40	Zero

*The dose to each group was divided into 5 fractions, delivered at 4-day intervals.

The results of this study (Table 2) show the following:

1. Radiation-induced damage in the mouse has one component which is permanent and irreversible.

2. At least a fraction of this irreversible damage is proportional to the magnitude of the challenging dose, and is measurable in terms of impairment in survival time in a continous gamma radiation field.

3. Fission neutrons are more effective in damaging the repair mechanism than are gamma rays by a factor of approximately 5.

References

(1) G. A. Sacher, Chapter 12, In: Radiobiology and Medicine W. Claus (ed.), Addison-Wesley, Reading, Mass. (1958).

(2) J. B. Storer, Rad. Res. 10, 180 (1959).

TABLE 2. Mean Accumulated Dose of Chronic Gamma Radiation Required to Produce Death Subsequent to Acute Gamma Ray and Fast Neutron Exposures

Group No.	Mice Surviving at End of 90-day Repair Period (No.)	Range (rads) Low	Range (rads) High	MAD (rads)	Controls (%)	Reciprocal
Gamma Rays						
1	42	2270	8857	5043	87.76	12.24
2	42	2090	8771	4825	83.97	16.03
3	41	644	6990	4109	71.51	28.49
4	22	890	8378	4029	70.11	29.89
5	10	3033	5992	3382	76.26	23.74
Neutrons						
6	42	2922	8141	4719	82.12	17.88
7	42	1152	6471	4231	73.32	26.68
8	42	134	5791	4059	70.64	29.36
9	36	839	6077	3074	53.50	46.50
10	11	644	2546	1752	30.49	69.51
Pooled Controls						
11	40	1976	8136	5746		
12	40	3510	8809			

Acute End Points as Indicators of Aging Induced by Radiation

(J. E. Furchner and G. A. Trafton)

Introduction

Premature aging or life shortening appears to be one of the important effects of radiation exposure. Normally, the aging effect is measured by comparing the median survival time of irradiated and control animals, which requires observation throughout the entire life span. Acute end points of radiation-induced aging would permit more observations with less handling and housing problems and would supplement median survival studies. It is possible also that such observations would contribute fundamental information on the mechanisms of radiation aging. This study was undertaken to explore calcium uptake and accumulation in the aorta, kidney hypertrophy after unilateral nephrectomy, and temperature shortening of the rat tail tendons as physiologic indicators of radiation-induced aging.

Methods and Results

Calcium Uptake by the Aorta

It has been shown (1) that calcium turnover in the aorta of rats increases with age. The chronic effects of radiation have been treated as accelerated aging (2). The present

experiment attempts to measure physiologic age and the effect of a single acute radiation dose on such a measurement.

Male Sprague-Dawley rats were irradiated with 350 rads of 250 KVP X rays at the ages of 3, 6, 9, and 12 months. Two months later the rats were injected intraperitoneally with about 2 mμ of Ca^{45}, as were unirradiated control rats of the same ages. The rats were sacrificed 24 hours after injection and the aorta, from the heart to the diaphragm, was removed. The aorta was assayed for total calcium and Ca^{45}. The results, expressed as per cent of injected dose per 100 mg of dry tissue weight, per cent of injected dose per μg calcium times 10^5, and μg calcium per mg dry tissue, are given in Figs. 1, 2, and 3, respectively.

The calcium content of the aorta seems to decrease between the ages of 3 and 12 months, while the Ca^{45} uptake doubles, indicating an increased turnover. The effect of an acute dose of 350 rads is negligible after 2 months. It is not known whether an effect would be detected at earlier or later intervals or whether larger radiation doses, delivered chronically, would demonstrate an effect.

Kidney Hypertrophy after Unilateral Nephrectomy in C57Black Mice

If the response of a young animal to a stress is more potent in relieving the resultant strain than that of an old

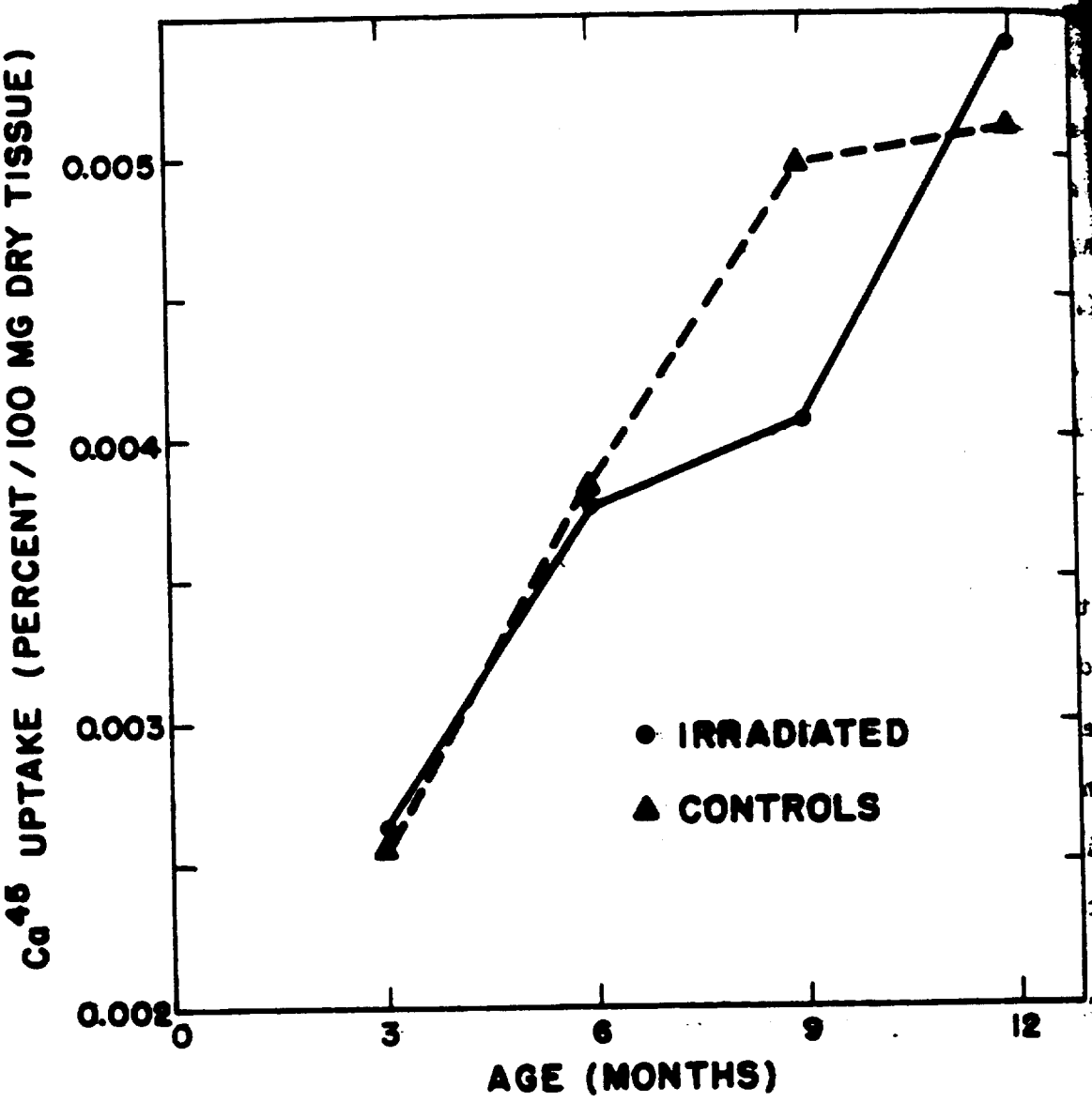

Fig. 1. Calcium^{45} uptake by aorta of the rat as a function of age and irradiation.

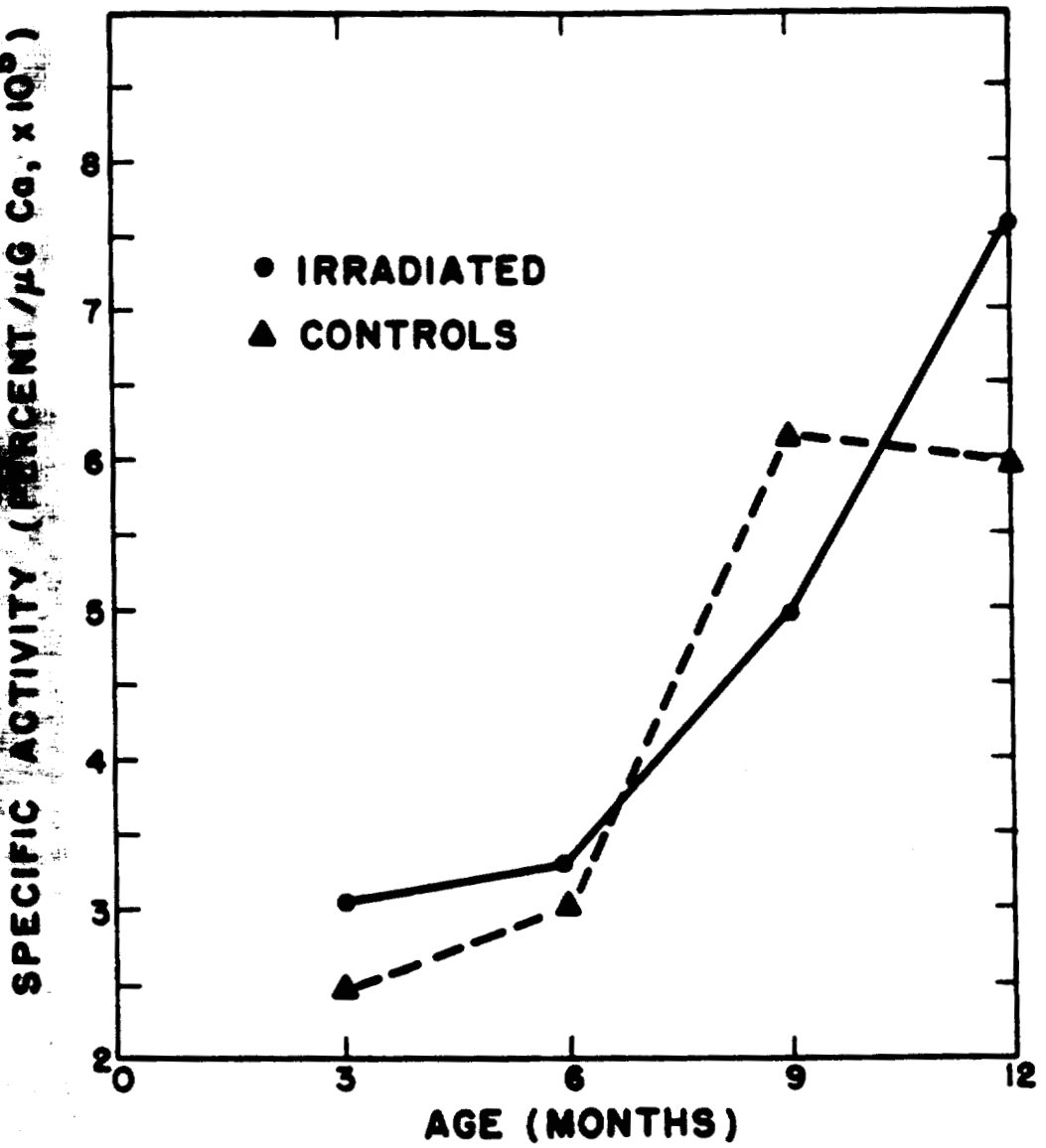

Fig. 2. Relative specific activity of calcium of the rat aorta as a function of age and irradiation.

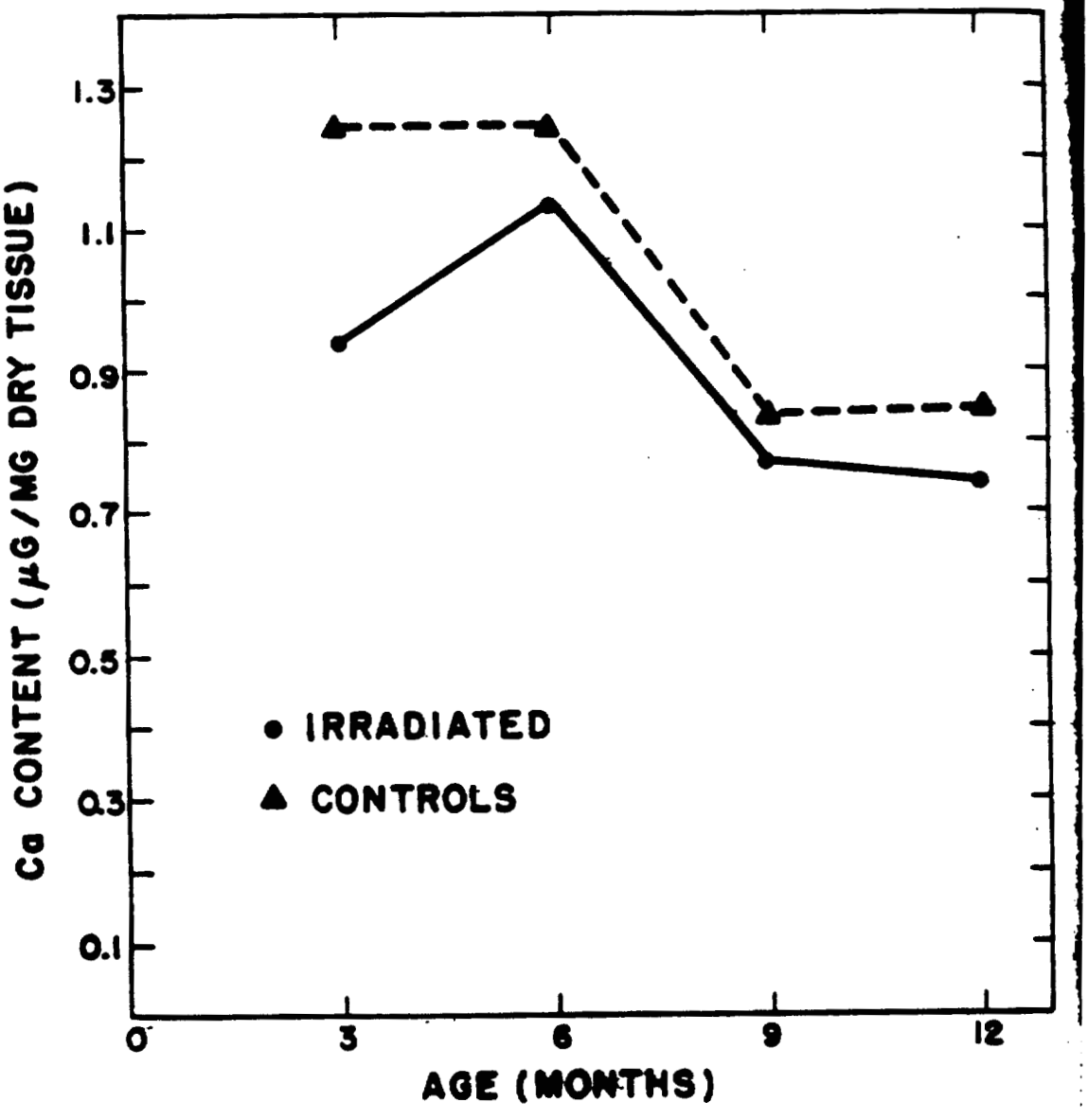

Fig. 3. Calcium content of the rat aorta as a function of age and irradiation.

animal, then the response to a stress may be a measure of physiological age. The hypertrophy of a surviving kidney is a well known response to the stress of unilateral nephrectomy. Several experiments testing this response as a function of age and radiation dose were carried out.

Acute Response to Radiation.--Twenty-four hours after exposures to X-ray doses of 186, 279, 372, 465, and 558 rads, groups of C57Black female mice were unilaterally nephrectomized, and the kidney was blotted dry and weighed. Forty days after nephrectomy, the animals were sacrificed and the surviving kidney was weighed. The per cent gain was calculated as follows:

$$\frac{\text{Kidney weight at sacrifice/Body weight at sacrifice}}{\text{Kidney weight at nephrectomy/Body weight at nephrectomy}} \times 100$$

The results are shown in Fig. 4. There was a decrease in hypertrophy linear with the logarithm of dose, except for the last point which may be due to a connective tissue hypertrophy at this dose in the animals in this group (35 per cent of the animals at this dose level died).

Hypertrophy as a Function of Age.--C57Black female mice were unilaterally nephrectomized at 3, 6, 9, 12, 18, and 24 months of age. Forty days after nephrectomy, the animals were sacrificed. Figure 5 shows the results. The last point

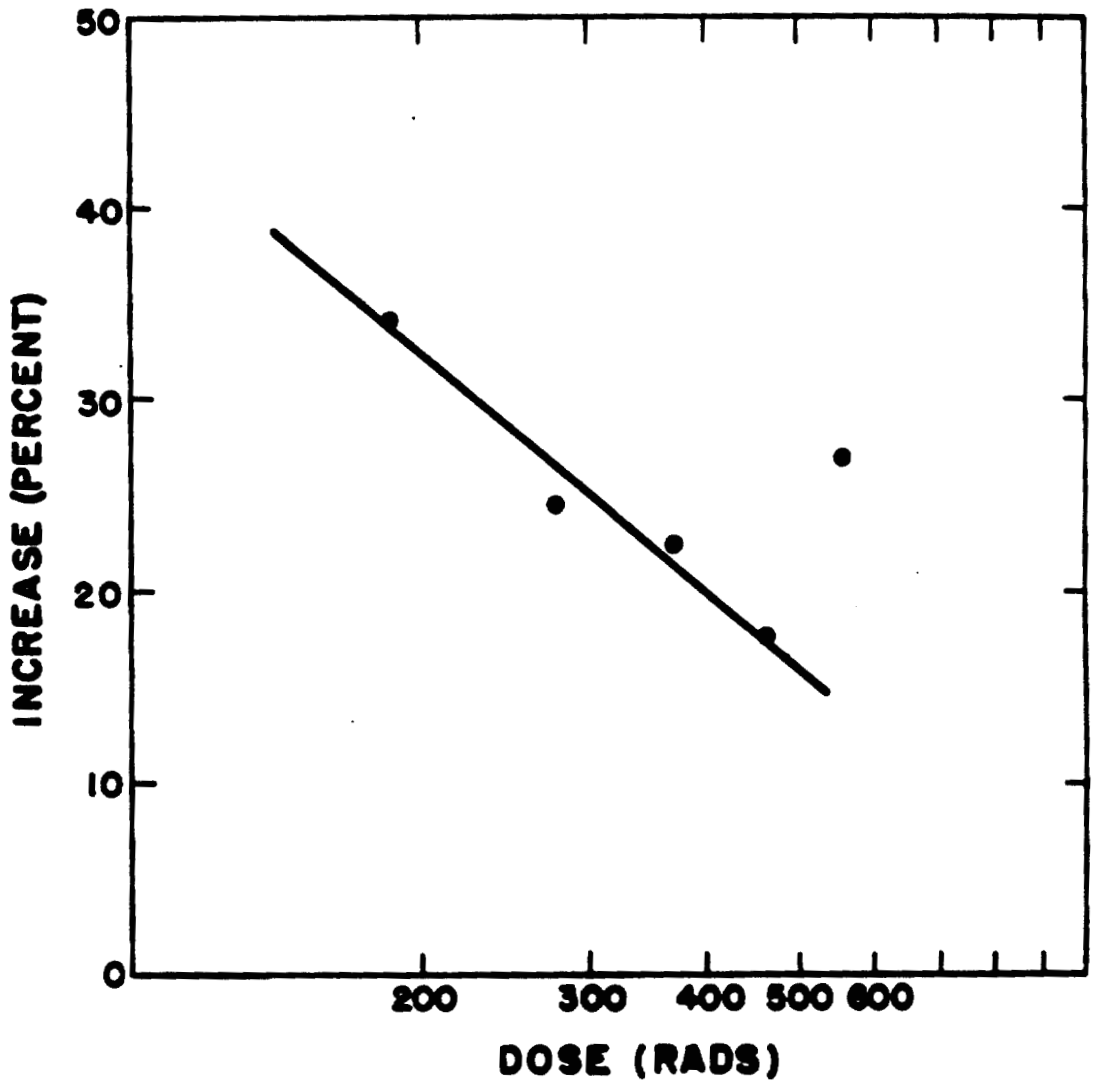

Fig. 4. Acute response of hypertrophying mouse kidney to irradiation.

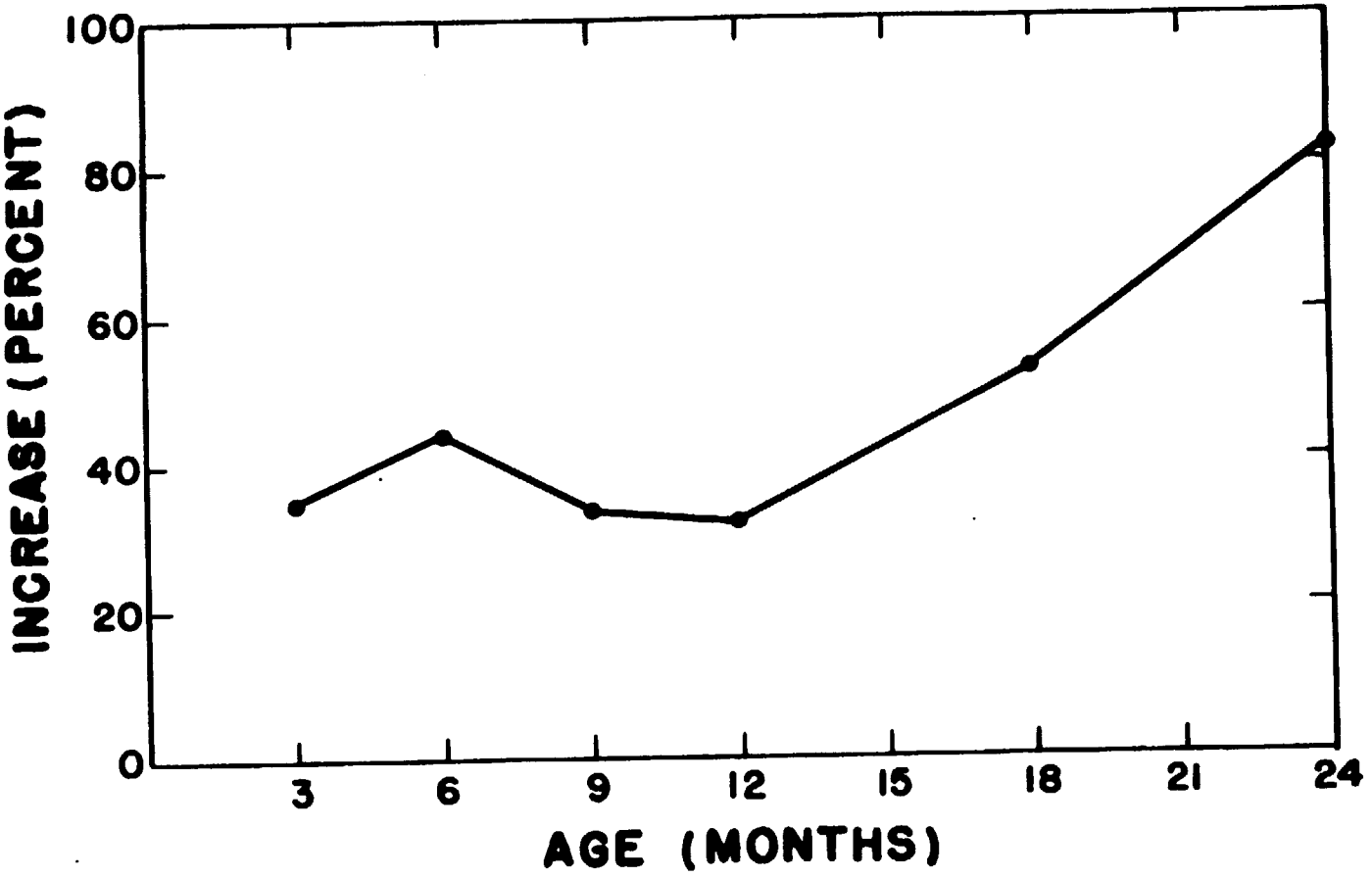

Fig. 5. Age dependence of hypertrophy of the mouse kidney following unilateral nephrectomy.

is of doubtful value, since only 4 animals survived 40 days post nephrectomy. It seems evident that between 3 and 18 months of age no useful relation between age and kidney hypertrophy was found. Verzar and Hugin found similar results in rats (3).

Delayed Effects of Radiation on Hypertrophy.--C57Black female mice were irradiated at the ages of 3 and 9 months with 200, 300, and 400 rads; a group of 3-month-old mice was also given 100 rads. Two months after irradiation, the mice were unilaterally nephrectomized. Forty days after nephrecto the mice were sacrificed. The results are given in Fig. 6 and give no particular indication that age of the animal at time of unilateral nephrectomy and irradiation has any effect on kidney hypertrophy as an indicator of radiation effect. Furthermore, there seemed to be no delayed response of kidney hypertrophy to irradiation.

Shortening of Rat Tail Tendons as a Function of Age.--Verzar (4) showed that the isolated tail tendons of rats shorten when exposed to a temperature of 60°C and that the amount of contraction is a function of temperature and age of the rat. An attempt to repeat this experiment was made on Sprague-Dawley rats 5 and 15 months of age. The results are given in Fig. 7.

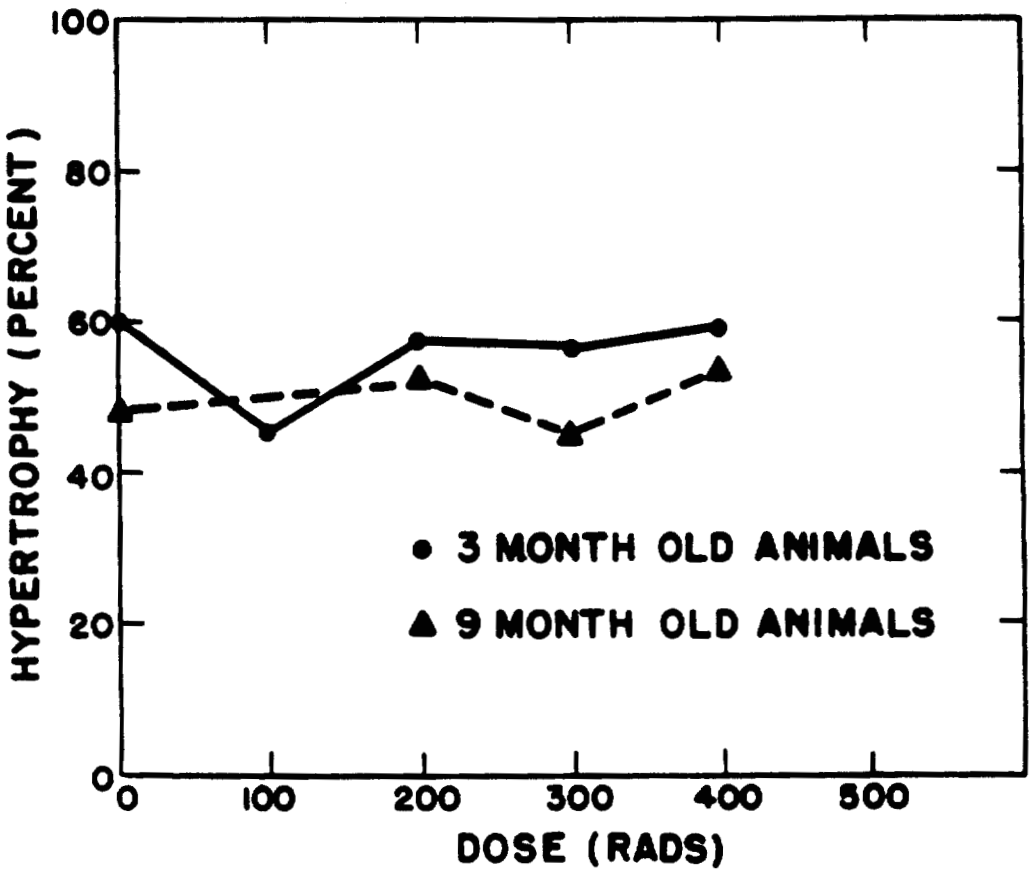

Fig. 6. Delayed response of kidney hypertrophy to radiation exposure in mice of different ages.

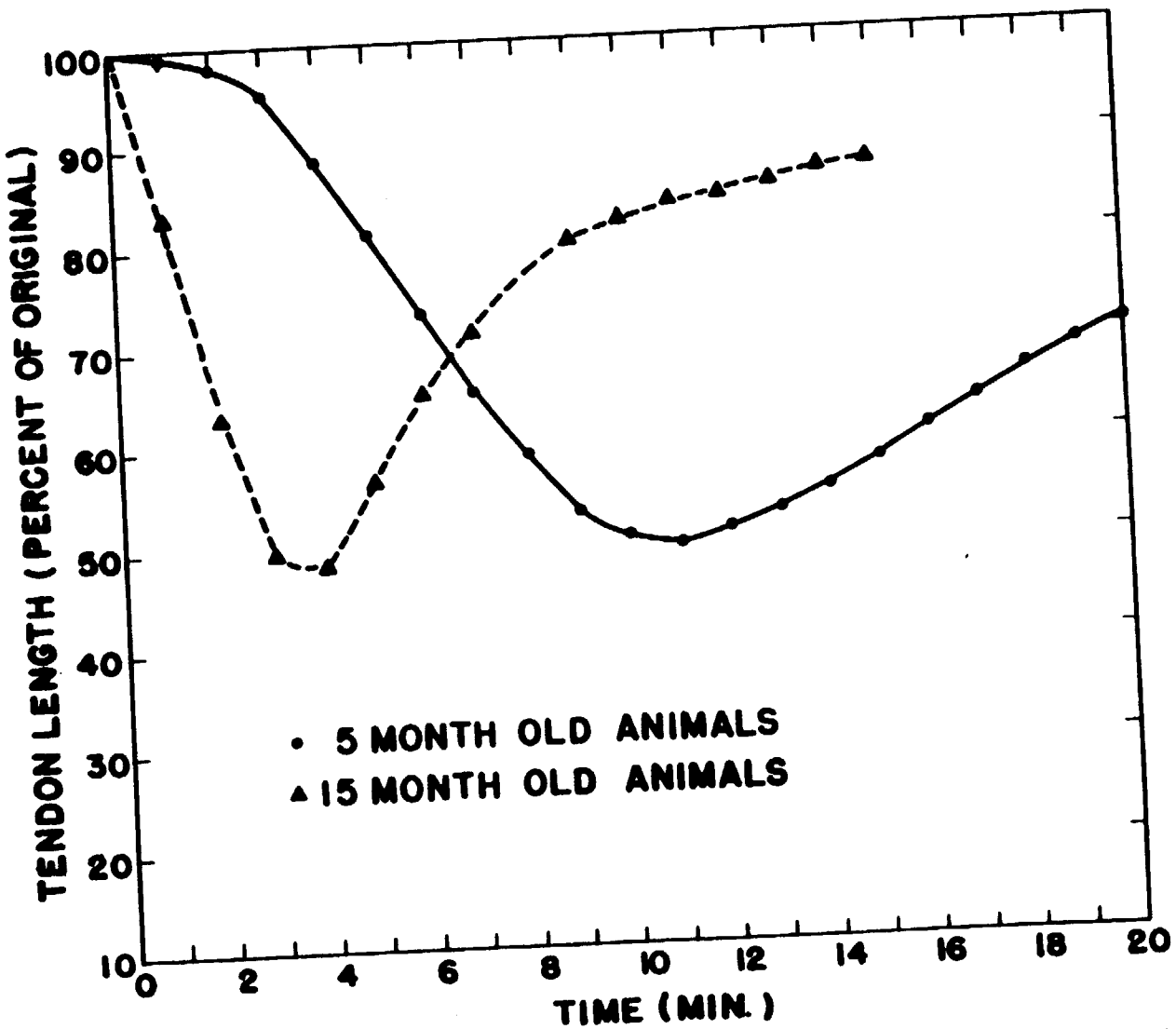

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Fig. 7. Age and time response of temperature shrinkage of the rat tail

It can be seen that the per cent shrinkage does not vary with age, although the time at which maximum contraction is reached is different at these ages. Further tests at other ages should be made. It is possible that the time of maximum contraction could be used as an indication of age response.

Conclusions

Although these studies were strictly preliminary, they seem to support the conclusion that the three methods tested show little promise as acute end points of radiation-induced aging. It is anticipated, however, that a search for such indicators will be continued.

References

(1) V. Freydberg-Lucas and F. Verzar, Experientia, Suppl. IV, 88 (1956).

(2) B. Strehler, Quart. Rev. Biol. 34, 117 (1959).

(3) F. Verzar and F. Hugin, Acta Anat. 30, 918 (1957).

(4) F. Verzar, Helv. Physiol. Acta 14, 207 (1956).

Dose and Flux Measurements on Godiva Radiation Effects Experiments (P. S. Harris, E. F. Montoya, and W. H. Schweitzer)

Introduction

The Los Alamos Godiva II assembly is used extensively as a radiation source for the study of radiation effects on biological materials, as well as effects on physical assemblies, electronic components, and other materials. It is especially used in experiments involving effects of fission neutrons. In such studies, it is important to know the dose and nature of the radiation exposure at the point of interest with the object being exposed in place. It was considered worthwhile, therefore, to make a study of radiation dose and flux from the assembly under a series of conditions of usage.

Methods and Results

A series of measurements on neutron flux, neutron dose, and gamma ray dose were made under several experimental conditions commonly used at Godiva when radiation effects experiments are being performed. Descriptions of the various experimental setups and conditions are not given here, since a document giving pertinent details (1) has already been prepared and is ready for distribution. The results indicate

the variations from free air estimates that might be expected under perturbing conditions. These experiments led to several conclusions of importance in radiation effects studies at this source, among which are the following:

1. Local environmental perturbations produced by the experimental objects themselves modify both fast neutron and gamma ray doses at the points of interest.

2. A monitor such as temperature rise in the assembly cannot be used to give an accurate index of either flux or dose at the position of some experimental object.

3. Sulfur as a flux or dose monitor cannot be used to give an accurate estimate of total flux or total dose at the position of some experimental object.

4. Plutonium surrounded by B^{10} can be used to give a reasonably accurate estimate of total fast flux and total fast neutron dose (accuracy better than 20 per cent) at the position of an experimental object under the usual conditions of the experiment. If the leakage spectrum is highly modified by the use of low Z interposed materials, plutonium in itself is unsatisfactory.

5. Gamma ray total dose cannot be predicted from a total neutron dose measurement in radiation effects experiments to better than a factor of 3 to 4.

6. Thermal neutron fluxes are not predictable and (if

of any importance) must be measured.

7. Gamma ray dose measuring devices should be thermal neutron insensitive because the presence of perturbing materials generally tends to raise the thermal neutron flux background and many standard gamma dose detectors have a high sensitivity (on a rad for rad basis) to thermal neutrons.

Reference

(1) P. S. Harris, E. F. Montoya, and W. H. Schweitzer, Dose and Flux Measurements on Godiva Radiation Effects Experiments. Los Alamos Scientific Laboratory Report LA-2355 (1959).

Effect of Sublethal Whole Body Irradiation at Different Age Levels (I. U. Boone, G. Trafton, L. Conklin, and D. C. White)

Introduction

Although acute and chronic effects of radiation on mice are accepted as being somewhat dependent on age at time of exposure, there have been few studies in which results have been collected in the same laboratory, under the same conditions, and on the same random population of animals. This study is an attempt to observe age dependence of several radiation effects under the same set of conditions controlled as carefully as possible.

Methods

CF_1 female mice from the same original random group were exposed to whole body radiation of 100, 200, and 400 rads at 2, 6, 12, and 18 months of age. Groups of animals exposed at 18 months of age contained 40 to 50 animals. All other irradiated groups had 150 animals. There were 300 animals in the control group. Also, from the same original random group, nonirradiated mothers were chosen for breeding purposes and their babies were exposed at 1 to 7 days of age. This part of the study includes males and females. Following irradiation, the babies were placed with their mothers for 30 days, weaned, and rerandomized. The number of animals in each group

varied between 80 and 100 at time of exposure.

Results and Discussion

The acute 30-day mortality for animals irradiated with 400 rads at 2 months of age or older is shown in Table 1. Tables 2 and 3 summarize the mean survival and life shortening of the animals irradiated at the various age levels. In Table 3, the life shortening was based on the mean survival of the control animals. Although only one control group of animals was used for the calculation of life shortening in Table 3, the life shortening of the irradiated group was based on the mean survival of control animals calculated on the basis of the number of control survivors at the time of irradiation of each of the irradiated groups. Animals irradiated in the first week of life were compared to their own set of control animals, as given in Table 1. The sensitivity to acute effects of irradiation appears to be increased with the age of the animal while the chronic delayed effects on life shortening decreased with age.

The total tumor incidence in these animals is given in Tables 4 and 5. Only the number of primary tumors occurring are reported. No specific breakdown in tumor classification is attempted in this report. All tumors of reticular tissue have been classified as leukemias and lymphomas, as

TABLE 1. Acute Mortality of CF_1 Female Mice Exposed to Whole Body X Irradiation at Various Age Levels

Age at Exposure (months)	Dose (rads)	Animals (No.)	Acute 30-day Mortality (per cent)
2	400	152	6.6
	Control	310	0.3
6	400	154	11.0
	Control	305	1.3
12	400	155	0.0
	Control	264	2.9
18	400	45	26.7
	Control	202	6.0

TABLE 2. Life Shortening Effect on CF_1 Mice Exposed to Whole Body X Irradiation at 1 to 7 Days of Age

Dose (rads)	Number Animals (females)	Mean Life Span (days)	Life Shortening (per cent)	Number Animals (males)	Mean Life Span (days)	Life Shortening (per cent)
100	84	580	3.0	97	585	8.7
200	93	426	28.8	95	428	33.2
400	52	372	37.8	45	355	44.6
Control	114	598	--	106	641	--

TABLE 3. Life Shortening Effect on CF_1 Female Mice Exposed to Whole Body X Irradiation at Various Age Levels

Age at Exposure (months)	Dose (rads)	Animals (No.)	Mean Life Span (days)	Life Shortening (per cent)	P-values
2	100	147	535	13.4	0.01
	200	145	459	25.7	0.01
	400	142	355	42.5	0.01
	Control	309	617	--	--
6	100	151	544	13.5	0.01
	200	141	532	15.4	0.01
	400	137	427	32.1	0.01
	Control	301	629	--	--
12	100	139	627	8.2	0.01
	200	139	629	7.9	0.01
	400	128	590	13.6	0.01
	Control	259	683	--	--
18	100	42	74	1.3	0.60
	200	45	712	5.7	0.02
	400	33	704	6.8	0.01
	Control	190	755	--	--

TABLE 4. Per cent Tumor Incidence in CF_1 Mice (irradiated at 1 to 7 days of age)

Dose (rads)	Animals (No.)	Leukemia and Lymphoma	Lung	Mammary	Ovarian	Others
Females						
100	63	15.9	15.9	1.6	25.4	4.7
200	74	20.3	9.6	2.7	18.9	2.7
400	43	39.5	9.3	0.0	18.6	2.3
Control	7	15.5	15.5	5.6	2.8	4.2
Males						
100	79	15.2	27.8	0.0	--	5.1
200	69	15.9	17.4	0.0	--	4.3
400	34	23.5	14.7	0.0	--	5.9
Control	80	12.5	18.8	0.0	--	5.0

TABLE 5. Per cent Tumor Incidence in CF_1 Female Mice Exposed to Whole Body X Irradiation at Various Age Levels

Age at Exposure (months)	Dose (rads)	Animals (No.)	Tumor				
			Leukemia and Lymphoma	Lung	Mammary	Ovarian	Other
2	100	116	17.2	17.2	3.4	17.2	3.4
	200	112	33.9	15.2	1.8	15.2	6.3
	400	102	32.6	10.8	1.0	5.9	3.9
	Control	236	18.6	18.6	3.4	3.0	1.7
6	100	119	12.6	14.7	4.2	6.9	2.6
	200	109	28.4	21.0	3.8	6.7	1.9
	400	112	19.6	13.0	4.0	2.0	1.0
	Control	233	18.9	18.9	3.4	3.4	1.3
12	100	101	16.8	21.0	3.0	6.0	0.0
	200	108	16.7	15.1	1.9	4.7	2.8
	400	113	16.8	15.4	1.0	2.1	4.1
	Control	206	20.9	21.4	3.4	3.4	1.9
18	100	43	37.2	25.6	2.6	0.0	5.1
	200	35	31.4	22.9	0.0	0.0	2.9
	400	39	17.9	27.6	0.0	0.0	6.9
	Control	156	20.4	25.6	1.9	4.5	2.6

recommended by Dunn (1). Pituitary tumors were not investigated. The occurrence of multiple primary tumors in one animal varied considerably between the groups of animals studied. Each primary tumor was considered separately.

In the irradiated babies, the leukemia incidence was increased in both sexes, but at higher doses the increase was greater in the females. Lung tumor incidence was increased slightly in the group of males receiving 100 rads. The number of ovarian tumors was greatly increased in the irradiated females. The threshold dose for ovarian production appeared to be less than 100 rads in this strain of mice. In general, where the over-all tumor incidence is increased over that of the control animals, the latent period for tumor induction is decreased.

The tumor incidence data for all mature age groups (Table 5) show that the leukemia incidence varies with age in this strain of mice. The greatest susceptibility is seen at 2 months and then at 18 months. Animals irradiated at 12 months did not show any increase in leukemia over that of the control animals. An increased incidence in ovarian tumors was seen in animals irradiated at 2 months of age. No increase in ovarian tumor incidence was seen in animals irradiated at 6 months of age or older. The over-all incidence of lung tumors did not differ from the control

incidence, but the latent period for production of these neoplasms is reduced in irradiated animals.

Gompertz function (age specific log rates of mortality) will be calculated for all groups of animals.

Reference

(1) T. B. Dunn, J. Nat. Cancer Inst. 14, 1281 (1954).

Effects of Partial and Whole Body X Irradiation on Life Span and Tumor Incidence of CF_1 Mice (I. U. Boone, G. Trafton, L. Conklin, and D. C. White)

Introduction

Partial body exposure to ionizing irradiation is a technique which has been used to study a wide variety of radiation effects. In the past it has had particular application in acute mortality studies, and only recently it has been applied to long-term and life shortening effects. No specific experimental data relating to life span and life expectancy after partial body exposure were available until a recent short paper by Kallman and Kohn (1).

The present experiment was designed for the purpose of studying the long-term effects of partial body exposure with specific reference to shortening of life span, tumor incidence, and age specific rates of mortality (commonly known as Gompertz functions). These results were compared to those from mice receiving equivalent integral doses durin whole body exposures and to unirradiated control mice.

Experimental Design

Approximately 1500 CF_1 80-day-old female mice, weighin from 19 to 27 grams, were used in the study. Animals were randomized 150 per group and earmarked as to group before

exposure. During exposure, the mice were anesthetized with sodium pentothal (91 mg/kg body weight).

Every attempt was made to shield uniformly the upper or lower portions of the body, with the tip of the xiphoid process arbitrarily chosen as the anatomical landmark. The partial body shields were hollow cylinders of 1/4 in. lead, lined on the inside with paraffin. The outside was covered with brass so that the cylinders could be machined to give a flat end or "side" to prevent the cylinders from rolling.

All mice received only a single X-ray exposure. A 250 KVP Maxitron X-ray machine was used. The pie-shaped exposure tray containing the animals was continuously rotated during exposures. The air dose was 52 r/min with a tissue dose of about 50 rads/min. Phantom dosimetry indicated that the tissue dose to the shielded areas was only 3 per cent of the total dose delivered to the exposed tissue.

The whole body exposure doses were 100, 200, and 400 rads, while animals which were shielded (upper or lower body) received 200, 400, or 800 rads. The total weight of the tissue exposed in each of the shielded groups was the same. Thus the integral doses were equivalent in these cases. In addition, the integral dose of shielded animals receiving 800 rads was equivalent to that given in whole body exposure of 400 rads; shielded 400-rad equivalent to whole body 200-rad, etc.

Animals were autopsied at death, and tissue samples were taken from approximately 60 per cent of the animals. The mean survival times, tumor incidence, and age specific log rates of mortality were determined.

Results and Discussion

Table 1 summarizes the acute 30-day mortality data. The values are higher than might be expected. Unfortunately, due to importing large numbers of animals to our laboratory at this time, a moderate Salmonella outbreak occurred. Animals involved in this study were well isolated and the disease was self-limiting. Very little evidence of it was seen after the first 30 days. Only 2 nonirradiated control animals died in the following 30 days. No further deaths occurred in the control group until 190 days after the irradiation date. Long-term data were not influenced by this early infection, as animals dying in the first 30 days were not included in the mean survival or death rate data.

The mean survival time and per cent of life shortening as compared to nonirradiated control animals are summarized in Table 2. The standard error of the mean ranged from ± 14 to ± 19. The P-values given in this table are compared to th control animals. In Table 3 the data are expressed in terms of the decrement in life span per 100 rads and per kilogram-r

TABLE 1. Acute Mortality of Partial and Whole Body X Irradiated CF_1 Mice

Treatment	Exposure Dose (rads)	Animals (No.)	Acute 30-day Mortality (per cent)
Upper body shielded	200	147	10.2
	400	148	12.9
	800	156	22.4
Lower body shielded	200	151	7.3
	400	158	13.9
	800	161	8.7
Whole body exposure	100	151	8.6
	200	146	18.5
	400	154	35.1
Control	---	158	6.3

TABLE 2. Mean Survival Time and Life Shortening of Partial and Whole Body X Irradiated CF_1 Mice

Treatment	Dose (rads)	Animals (No.)	Mean Life Span (days)	Life Shortening (per cent)[b]	P-values[c]
Upper body shielded	200	132	567	6.9	0.05
	400	129	506	16.9	<0.01
	800	121	491	19.4	<0.01
Lower body shielded	200	140	588	3.5	0.10
	400	136	559	8.2	0.02
	800	147	549	9.9	<0.01
Whole body exposure	100	138	549	9.9	<0.01
	200	120	496	21.3	<0.01
	400	100	443	27.3	<0.01
Control	---	148	609	---	----

(a) Mice that died in the first 30 days after exposure were not included in the data.

(b) The standard error from the mean ranged from ± 14 to ± 19.

(c) p-values <0.05, as determined by "t" testing, were consider to indicate a significant difference from the control animals.

TABLE 3. Decrement in the Life Span per Unit Dose of Partial and Whole Body X Irradiated CF_1 Mice

Treatment	Dose (rads)	days/100 rads	day/kg-rad
Upper body shielded	200	21	18
	400	26	22
	800	15	13
Lower body shielded	200	11	9
	400	13	11
	800	8	6
Whole body exposure	100	60	26
	200	57	24
	400	42	18

For whole body exposure, the increased sensitivity of female mice as dose decreases in the 600 to 200 rad range has been noted and mentioned previously by Furth (2) and Sacher (3). Kallman and Kohn (1) have reported similar results. It has been suggested that this is somehow related to the peculiarly greater sensitivity to X rays of the mouse ovary.

A brief summary of the over-all tumor incidence is presented in Table 4. In this particular study, no striking over-all leukemia incidence was seen. The leukemia incidence may have been influenced by the Salmonella infection present at the time of irradiation. Thymic involution may have been present at the time of irradiation as a result of the infection. If the tumor incidence is examined on the basis of age intervals, the latent period for tumor occurrence is decreased in irradiated animals.

Age specific log rates of mortality for all causes of death, except leukemia, were obtained for all control and irradiated groups. In all instances, the regression coefficient, i.e., the slopes, did not differ significantly from that of the control and the lines could be drawn parallel as shown in Fig. 1. The Gompertz functions are displaced upward from the control in all instances. The most severe displacement occurred following 400 rads whole body exposure. Death rates following 800 and 400 rads to the lower half of the body could be represented by one line. Displacement following

TABLE 4. Per cent Tumor Incidence in Partial and Whole Body X Irradiated CF_1 Mice

Treatment	Animals (No.)	Dose (rads)	Tumor				
			Leukemia and Lymphoma	Lung	Mammary	Ovarian	Other
Upper body shielded	101	200	16.8	17.8	5.0	19.8	10.9
	97	400	17.5	19.6	3.1	13.4	9.3
	95	800	9.5	15.8	4.2	14.7	10.5
Lower body shielded	113	200	21.2	19.5	2.7	6.2	3.5
	105	400	28.6	23.8	1.9	2.9	2.9
	109	800	13.8	25.7	3.7	6.4	8.3
Whole body exposure	107	100	17.8	16.8	4.7	19.6	2.8
	89	200	16.9	16.9	2.3	13.5	2.2
	76	400	23.7	15.8	2.6	6.7	2.6
Control	119	---	23.5	18.5	4.2	2.5	4.2

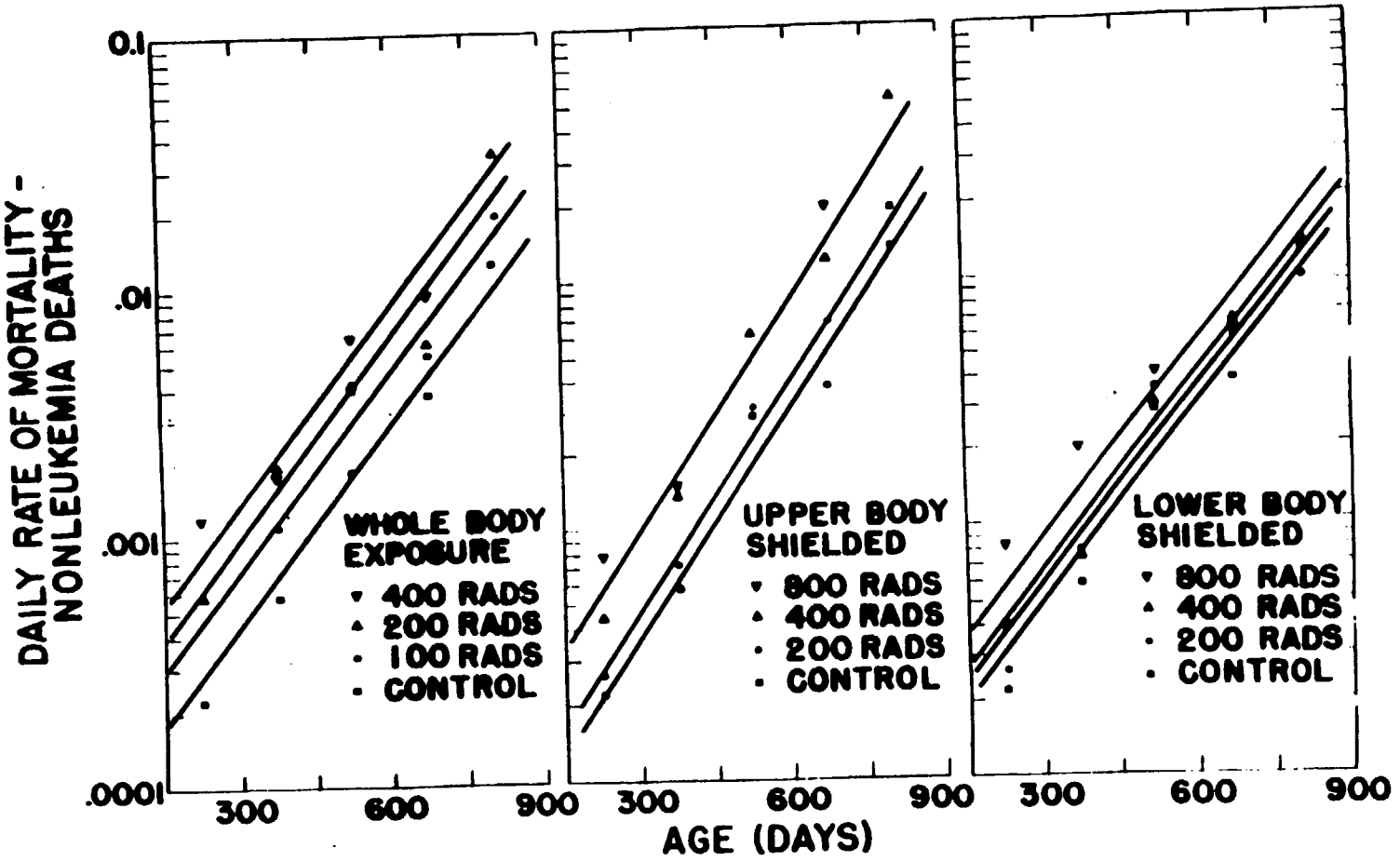

Fig. 1. Age specific rates of mortality for nonleukemia deaths. Age of exposure was about 80 days for all mice.

upper body exposure is not as great as that following lower body exposure. A plot of the displacement above the control group is given in Fig. 2.

Life shortening effect in partially exposed CF_1 mice was not strictly proportional to exposure or integral dose. The decrement in life span per unit dose was not constant. To understand the differences in the results between upper and lower body exposures, each must be considered on the basis of the particular lesion involved. The greater sensitivity following lower body exposures is no doubt influenced by the radiation sensitivities of the ovaries and the gastrointestinal tract.

References

(1) R. F. Kallman, and H. I. Kohn, Science 128, 301 (1958).

(2) J. Furth, and M. C. Boon, Science 98, 138 (1943).

(3) G. Sacher, Radiology 67, 250 (1956).

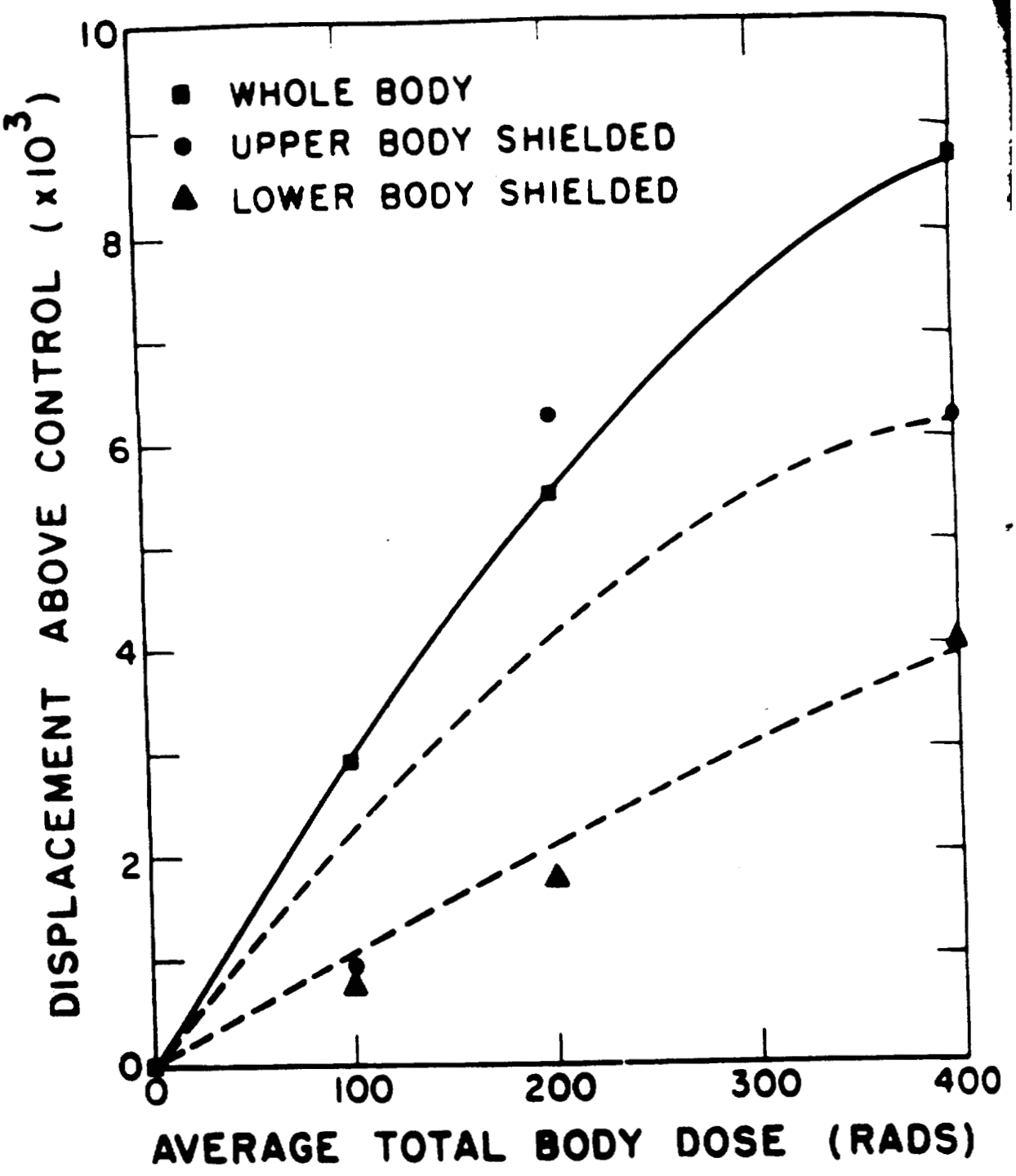

Fig. 2. Relation between displacement of Gompertzian intercept and dose.

Effect of Single Sublethal Doses of Nitrogen Mustard (HN_2) on Life Span and Tumor Incidence as Compared to Whole Body X Irradiation (I. U. Boone, G. Trafton, L. Conklin, and D. C. White)

Introduction

Many drugs used in cancer chemotherapy have been reported to produce biological effects similar to those produced by irradiation. One of the most observed effects of such drugs is that of production of mutations. For these reasons, such drugs are frequently called radiomimetric or mutagenic agents and are of considerable interest to radiobiologists. This study was designed to investigate the long-term and delayed effects of nitrogen mustard (HN_2) as compared to sublethal doses of whole body irradiation.

Methods and Results

Nitrogen mustard was administered as single sublethal doses to CF_1 and CFW Swiss female mice. The doses administered were chosen on the basis of LD_{50} studies conducted on each strain. The radiation was delivered as a single whole body dose of 400 rads of 250 KVP X irradiation. The air dose rate was 52 r/min with a tissue dose rate of 50 rads/min. The CF_1 mice were irradiated or treated at about 4 months of age, and the CFW Swiss mice at about 3 months of age.

The data showing acute 30-day mortality from nitrogen mustard are given in Table 1. Table 2 summarizes the mean survival and life shortening data for both radiation and HN_2, and data showing tumor incidence are presented in Table 3. While nitrogen mustard did not significantly affect the life span of CF_1 mice, 400 rads of whole body X irradiation shortened the mean life span of CF_1 mice by 38 per cent. In this strain of mice, the leukemia incidence was definitely elevated only in the group exposed to 400 rads. Nitrogen mustard (4 mg/kg) did not influence the leukemia incidence but did increase the incidence of lung tumors. The increase in lung tumors may not have been reflected in the life span data, as this tumor has a long latent period even in the mice receiving HN_2. Ovarian tumors were only slightly increased in the irradiated CF_1 mice. This may be related to the age at which the animals were irradiated, which was about 4 months in this case.

In the CFW Swiss strain of mice, life shortening following 400 rads of whole body irradiation was only 13.7 per cent. Mice of this strain which received 2.5 mg/kg of HN_2 showed no significant life shortening effect, while mice receiving 3.5 mg/kg of HN_2 had a reduction in mean life span of 9.2 per cent.

The reduction of life span of animals in the 400 rad

TABLE 1. Acute 30-day Mortality in CF_1 and CFW Swiss Female Mice following Administration of HN_2 or X Irradiation

Treatment	Animals (No.)	Acute 30-day Mortality (per cent)
CF_1 Mice		
4 mg/kg HN_2	138	1.4
400 rads	147	4.1
Control	141	0
Swiss CFW Mice		
3.5 mg/kg HN_2	91	2.2
2.5 mg/kg HN_2	87	3.2
400 rads	97	1.0
Control	111	0.9

TABLE 2. Life Shortening Effect in CF_1 and CFW Swiss Female Mice following Administration of HN_2 and X Irradiation

Treatment	Animals[a] (No.)	Mean Life Span (days)[b]	Life Shortening[c] (per cent)	P-values[d]
CF_1 Mice				
4 mg/kg HN_2	136	610	3.1	0.40
400 rads	141	391	37.9	< 0.01
Control	141	630	---	--
Swiss CFW Mice				
3.5 mg/kg HN_2	89	542	9.2	0.02
2.5 mg/kg HN_2	86	578	3.2	0.50
400 rads	87	515	13.7	< 0.01
Control	110	597	---	--

(a) Mice that died in the first 30 days after exposure were not included in the data.

(b) Standard error from the mean ranged from $\pm$ 13 to $\pm$ 18.

(c) Relative to controls.

(d) P-values $\leq$ 0.05, as determined by "t" testing, were considered to indicate a significant difference from the control animals.

TABLE 3. Per cent Tumor Incidence in CF_1 and CFW Swiss Female Mice following Administration of HN_2 and X Irradiation

Treatment	Animals (No.)	Tumor				
		Leukemia or Lymphoma	Lung	Mammary	Ovarian	Other
CF_1 Mice						
4 mg/kg HN_2	110	20.0	39.1	1.8	7.3	0.9
400 rads	112	39.3	9.8	2.7	7.1	1.8
Control	118	26.3	20.3	5.1	5.9	2.5
CFW Swiss Mice						
3.5 mg/kg HN_2	68	32.4	25.0	8.8	0.0	2.9
2.5 mg/kg HN_2	72	23.6	20.8	9.7	2.8	5.6
400 rads	55	34.6	12.7	3.6	1.8	7.3
Control	77	20.8	16.9	11.7	0.0	1.3

X irradiated and 3.5 mg/kg HN_2 groups may be related to the leukemia incidence. Incidence of leukemia was increased in both of these groups, and the latent period for leukemia induction was decreased. The incidence of lung tumors was only slightly increased in the HN_2 groups and unaffected by the radiation. Mammary tumor incidence in irradiated mice was lower than that of the control mice. Neither the 400 rad of whole body X irradiation nor the HN_2 induced ovarian tumor formation in this strain.

Effect of Pre-irradiation Treatment with Glutathione on Life Span and Tumor Incidence of CF_1 Mice (I. U. Boone, G. Trafton, L. Conklin, and D. C. White)

Introduction

Agents such as cysteine, cysteamine, glutathione, S,2-aminoethylisothiuronium dibromide (AET), etc., administered before irradiation have been shown to exert a protective effect by modifying the response of acute lethal doses of X irradiation. In this study, glutathione (a known protective agent) was administered to CF_1 female mice prior to a single lethal or sublethal dose of X irradiation. Long-term effects on life span, tumor incidence, and age specific log rates of mortality were compared to animals receiving a single dose of 400 rads of whole body X irradiation and to nonirradiated animals.

Methods and Results

The radiation was delivered as a single dose of 250 KVP X irradiation. The air dose was 52 r/min with a tissue dose of 50 rads/min. Glutathione (4 g/kg body weight) was administered 30 minutes before irradiation. The mice were treated and irradiated at 17 weeks of age. Table 1 lists the groups, the number of animals in each group, and the acute mortality data. Table 2 summarizes the mean survival and life shortening data. The decrement in life span per unit is given in

TABLE 1. Acute 30-day Mortality in Irradiated CF_1 Mice Pretreated with Glutathione

Treatment	Animals (No.)	Acute 30-day Mortality (per cent)
400 rads	147	4.1
Glutathione + 400 rads	141	0.7
Glutathione + 700 rads	142	6.0
Glutathione only	154	0
Control	141	0

TABLE 2. Mean Survival Time and Life Shortening in Irradiated CF_1 Mice Pretreated with Glutathione

Treatment	Animals (No.)	Mean Life Span[a] (days)	Life Shortening[b] (per cent)	P-values[c]
400 rads	141	391	39.8	< 0.01
Glutathione + 400 rads	140	479	26.2	< 0.01
Glutathione + 700 rads	134	427	34.2	< 0.01
Control I (glutathione)	154	666	--	0.10
Control II (no glutathione)	141	630	--	--
Combined control Groups[d]	295	649	--	--

(a) Includes time prior to radiation exposures but does not include data for mice dying in the first 30 days after exposure.

(b) Relative to combined control Groups I and II.

(c) P-values ≤ 0.05, as determined by "t" testing, were considered to indicate a significant difference from the control animals which did not receive glutathione, i.e., control Group II.

(d) Combined control groups, i.e., animals which received glutathione and those receiving no glutathione.

Table 3. The over-all tumor incidence is summarized in Table 4.

Glutathione had a definite protective effect in terms of life shortening and tumor incidence at both the lethal and sublethal dose levels. Age specific log rates of mortality for nonleukemia and all deaths have also been calculated for these groups.

TABLE 3. Decrement in Life Span per Unit Dose[a] in X Irradiated Mice Pretreated with Glutathione

Treatment	Day/100 rads	Day/kg-rads[b]
400 rads	65	26
Glutathione + 400 rads	43	17
Glutathione + 700 rads	32	13

[a]Based on combined control groups of animals with mean life span of 649 days.

[b]Average weight of mice used was 25 gm.

TABLE 4. Per cent Tumor Incidence in X Irradiated CF_1 Mice Pretreated with Glutathione

Treatment	Animals (No.)	Tumor				
		Lymphoma and Leukemia	Lung	Mammary	Ovarian	Other
400 rads	112	39.3	9.8	2.7	7.1	1.8
Glutathione + 400 rads	118	28.0	15.3	5.1	11.0	4.2
Glutathione + 700 rads	104	26.9	18.3	2.9	6.7	4.8
Control (glutathione only)	128	25.0	24.2	3.9	2.3	2.3
Control (no glutathione)	118	26.3	20.3	5.1	5.9	2.5

Neoplasms Occurring in a Series of Irradiated Mice (D. C. White)

Introduction

The scope of this study was to enumerate the tumor types noted in mice used in a variety of radiation experiments presented in the previous papers. The completed study will include a correlation of these tumor types with dose of radiation administered and the mode of administration, and will encompass the incidence of each variety of neoplasm.

Materials

To provide a more comprehensive evaluation of these experiments, a careful microscopic examination was made of all tissues removed at the time of autopsy. A total of 5,277 CFW mice were employed in the course of these several experiments, of which 3,333 (or about 60 per cent) were autopsied and tissues taken.

Of the total number of mice utilized, 960 were controls and received no irradiation. The remainder were administered doses of X irradiation ranging from 100 to 800 rads at various age levels with or without partial body shielding. Unless otherwise indicated, the following tissues were routinely removed for histologic examination: lungs, liver, spleen, kidneys, and sternum. Obvious lesions noted in other organs

or tissues were also preserved. No gross examination of the brain or spinal cord was made, nor was the skeletal system surveyed in any detail.

Classification

Without including actual percentages, the four most frequent tumor types noted in this study were pulmonary, mammary, ovarian, and reticulo-endothelial, including the leukemias and lymphomas.

Pulmonary

There is a rather marked variation in incidence of both spontaneous and induced pulmonary neoplasms among the numerous strains of mice currently used in biological research (1-4). This incidence varies from 80 to 90 per cent in strain A to relative resistance in the C57Black and L strains. The pathogenesis and morphology, however, remain unaffected by differences in strain or mode of induction.

There was no predilection as to site of origin except that the majority of these tumors apparently arise near the lung periphery. In general, those tumors arising spontaneously are solitary in contrast to the multicentric nature of those induced through chemical or physical means. These tumors grow primarily with a spherical configuration with progressive compression of encircling lung tissue. Not infrequently,

however, the adjacent alveoli are progressively invaded by cords of tumor cells extending along the septa. In these instances, the pseudoencapsulation noted in the expanding type of growth is relatively inconspicuous.

Microscopically, the basic structure of these pulmonary tumors is similar. There is a spectral variation in the cellular pattern based on tumor location, amount of supporting stroma, etc. The pattern is that of an epithelioid tumor showing a predominance of acinar and papillary configurations. The tumor cells are cuboidal or low columnar. The cytoplasm is uniformly homogeneous and slightly acidophilic. Occasional tumors are observed in which the cells exhibit paranuclear vacuoles. The free borders of these cells show no evidence of cilia formation. The nuclei are, in general, uniformly ovoid or spherical in form. The relative size of the nuclei, as well as the degree of staining, may vary from tumor to tumor and, in fact, from area to area in a single tumor. This inconsistency gives these tumors a variegated appearance. This may also become evident when two tumor foci coalesce in the course of their growth.

With the exception of the relatively infrequent anaplastic tumor forms, the number of mitotic figures is extremely small. Despite the essentially benign appearance of these tumors, however, they must of necessity be considered as malignant or

potentially malignant by virtue of their mode of growth and transmissibility. A unique and rather striking feature of these tumors is the morphologic transformation which occurs when one of these tumors breaks through the pleura or metastasizes to regional lymph nodes. The tumor then assumes a sarcomatous pattern with only scattered attempts at acinar formation. Distant metastatic lesions must be extremely rare, as none was noted in this study.

Grady and Stewart (1), in a detailed analysis of the histogenesis of these tumors; utilized proven methods of rapid tumor induction to study the site of origin. This was accomplished by the daily sacrifice of several animals and serial sectioning of the fixed lung tissue. The results of this experiment and a similar study performed by Mostofi and Larsen (5) showed conclusively that these tumors arose in alveolar tissue with no apparent association with bronchioles or bronchi. This was of great importance as the cellular morphology of these tumors would, in many instances, suggest an origin from bronchiolar epithelium. It is still conceivable that they may stem from "multipotential" cells at or near the bronchiolar-alveolar duct junction.

One other form of pulmonary tumor was occasionally noted in this series. Portions of the above tumors in rare instances exhibited definite squamous characteristics even to the formation of epithelial pearls. It could not be determined whether

this represented a true tumor variation of squamous metaplasia in the already existing neoplasm. The fact that this particular configuration always appeared as an integral part of the standard pulmonary tumor lends strong support to the theory of metaplasia.

Mammary

With but few exceptions all mice employed in these experiments were female; therefore, mammary tumors comprised a large percentage of the total number of neoplasms noted at autopsy. In order to maintain consistency with other similar studies on these tumors in mice, the classification of Dunn (6) was followed as closely as possible.

Mammary tissue in the female mouse may be found over a very broad area, extending from the cervical region to the vulva on the ventral surface and almost to the midline on the dorsum of the body. Because of this widespread distribution, any subcutaneous tumor arising in this area must be considered as potentially mammary in origin. In general, the gross characteristics of these tumors are as follows: rounded or ovoid, sometimes nodular, well circumscribed, moderately firm growths which on cut surface are seen to be composed of grayish-white tissue. Various additional features may at times be noted such as distended vascular channels, blood filled cysts, areas of necrosis, and an exudate of milky fluid.

With relatively few exceptions, these mammary tumors may be classified into three basic types on a morphologic basis. It must be stressed at this point that any attempt at delineating these tumors must be determined by the predominant histologic pattern. A pure tumor type is rarely noted as the neoplasm is generally an admixture of types and variations of the morphology are the rule rather than the exception.

Adenocarcinoma, Type A.--The predominant pattern is one of small follicles or tubules arranged generally in a lobular fashion. The lining of the follicles consists of a single layer of uniform cuboidal cells with homogeneous, slightly acidophilic cytoplasm. An occasional vacuole may be noted in the cytoplasm. The nuclei are small and ovoid or rounded with uniform chromatin distribution. Mitotic figures are infrequent, although this tumor type appears to metastasize as often as do the other varieties.

Adenocarcinoma, Type B.--Although there is a basic glandular structure present, there is considerable variation in the patterns which the epithelial cells assume. In general the cells are arranged in tubular- or cord-like fashion or in irregular acinar configurations, some of which display epithelial papillary formations. The lining epithelial cells exhibit variations in size and staining quality and fail to

show the uniform continuity found in Type A tumors. If the tumor pattern is such that a lumen exists, the free inner border is irregular and occasionally papillary. The nuclei are as variable as the cell morphology, and hyperchromatism with chromatin clumping is not unusual. A double cell lining is often a prominent feature in this tumor type with the more peripheral layer composed of somewhat elongated, deeply staining cells. These may be likened to myoepithelial cells.

A variant of this Type B tumor is worthy of note and might be considered as a separate entity. In some tumors examined, it has been found to compose a large portion of the total tumor mass. In this variant, there has been an extensive overgrowth of epithelial cells such as to form a solid mass of closely packed cells. In this particular configuration there is little, if any, predilection toward lumen formation. In the central regions of these cell masses, there are often cleft-like spaces, and it may occasionally be noted that the lumenal layer of cells has lined up with a definite polarity and in a relatively orderly fashion. The spaces thus formed contain an exudate which is frequently filled with sloughed cells and cell debris.

Adenoacanthoma.--Tumors given this specific designation must show a substantial portion of the tumor to have an epidermoid structure. The remainder of the tumor is either

Type A or B, or an admixture of both. Many of the glandular type tumors exhibit small foci of squamous elements as integral portions of the glandular structures. These foci appear to be examples of squamous metaplasia and do not seem to have any inherent neoplastic potential. In the adenoacanthomas, on the other hand, increased numbers of mitotic figures and a more disorganized growth pattern indicate true neoplasia.

Those tumors designated by Dunn as adenocarcinoma, Type C, were not noted in this series and may be peculiar to older mice of certain strains as is suggested by this author. Similarly, true molluscoid forms of adenoacanthoma were not found. All types of mammary tumors must be considered potentially malignant as are the pulmonary tumors. Metastasis to the lung and regional lymph nodes is not unusual, and direct extension to the adjacent connective tissue and muscle components is seen with great regularity.

Ovarian

Ovarian tumors are relatively uncommon in control mice, whereas irradiation increases the per cent tumor formation several fold. In an analysis of ovaries following irradiation, it is essential to be able to recognize the structural alterations produced by this injurious agent. There is a fi

differentiation between the sequence of events following ionizing radiation and initiation of a true neoplastic process. Following irradiation the ova degenerate, forming nests of granulosa cells in the cortex. Some of these nests are compact, whereas others are composed of granulosa cells loosely embedded in a colloid-like material. A progressive morphologic change takes place with these "follicular" cells becoming larger with an abundance of pale cytoplasm. In time, a definite hyperplasia of these elements may become evident with granulosa cell outgrowths into the adjacent ovarian stroma. The stroma remains essentially unaltered.

Of particular interest is the rather late occurrence of downgrowth of the germinal epithelium. These assume the characteristics of epithelial tubules, which often extend deeply into the ovarian tissue. This particular phenomenon is peculiar to mice that have been subjected to irradiation and is not found among the controls.

From these observations, it is apparent that the vast majority of tumors arising in irradiated mice must stem from the persisting "follicle" cells and the stroma, or the newly formed tubular downgrowths.

Granulosa-cell Tumors.--Although considered to be an uncommon tumor in the human female, it has the highest incidence among the ovarian tumors of irradiated mice. It is composed

of cells apparently morphologically related to those of th ovarian follicles. It is impossible to assign any specifi growth pattern to these cells, as they may appear as large solid compact masses, small nests resembling anovular follicles, cell cords, or gland-like structures. The amount of stroma may also display considerable variation. The so-called luteomata should be considered a variant of the gra ulosa cell tumor and consists of large, pale staining cells containing much lipid material in the cytoplasm. Only rare may one see a tumor composed entirely of such cells. A gradual shading from the typical granulosa cell to the full luteinized cell is the rule, and distinct areas of both cel types are generally noted in the same tumor. Another less common variant is the tumor which shows a definite spindle cell component, probably derived from the theca interna cells.

Tubular Adenoma.--The formation of these tubular down-growths has already been noted. The ovaries often remain o normal size, even though markedly honeycombed by these tubu It is pure conjecture as to whether an ovary of this nature represents true neoplasia or an atypical involutional respo to radiation. Not infrequently, however, such growth conti with enlargement of the ovary and true adenomatous tumor fo tion. In direct contrast to the granulosa cell tumor, ther

has been no detectable evidence of hormonal activity in these adenomas.

Simple cysts of the ovary will be mentioned in passing only because they probably represent the effect of an abnormality of ovarian function or residuum of hemorrhage and have no specific relation to neoplasia. In order to complete the classification of tumors noted in this study, certain less common tumors should be mentioned such as papillary cystadenoma and its rare malignant counterpart, papillary cystadenocarcinoma, teratoma, and embryonal cell carcinoma.

Reticulo-endothelial Neoplasms

As is the case with all tumors found in the mouse, a concerted effort has been made to parallel as closely as possible the classification of reticulo-endothelial neoplasms in man. Dunn's classification (6) is most comprehensive and complete. It is soundly based upon critical review of previous reports, careful evaluation of pathologic description, and an exhaustive survey of tissue preparations from her own laboratory. The classification is predicated upon the contention that these neoplasms originate from stem cells, granulocytes, lymphocytes, reticulum cells, and plasmocytes. This present study shall, therefore, have as its basis the classification as set forth by Dunn and her associates (7).

In the experiments from which this pathology survey emanated, the exact differentiation of these various reticulo-endothelial neoplasms was not considered to be essential to the projected goals. This report, therefore, is based entirely upon tissue sections without the benefit of peripheral blood or bone marrow preparations. The following types were encountered in this study.

Stem-cell Leukemia.--This is a rapidly progressing tumor with the cells appearing in the peripheral blood at an early stage and a very widespread distribution of organic lesions. The cells are relatively large and immature in morphology, with pale cytoplasm and large hyperchromatic nuclei which are frequently indented.

Lymphosarcoma.--This term is restricted to those lymphocytic neoplasms which remain localized for extended periods with no dissemination of cells into the peripheral blood. More than one organ may be involved in this entity. One of the most common representations of this tumor is lymphosarcoma of the thymus. This tumor may remain wholly within the confines of the capsule. There is, however, an eventual breakthrough with subsequent extension to the parasternal region and to the hilum of the lung, where perivascular and peribronchial "cuffing" become a prominent feature. Even with this extensive local spread, there may be no evidence of

systemic dissemination or invasion of the peripheral blood.

Lymphatic Leukemia.--This neoplasm may arise as an entity unto itself or stem from the eventual generalized dissemination of a pre-existing lymphosarcoma. The degree of systemic involvement may be extremely variable, but the majority exhibit "classic" generalized leukemic spread.

Granulocytic Leukemia.--As the mice being considered in this study were allowed to die from "natural" causes, the diagnosis of this entity was particularly difficult. Although a considerable per cent of these animals had large neoplasms of various types, the basic cause of death was primarily a massive pneumonia or pneumonitis with or without a histologically evident septicemia or bacteremia. These processes were invariably accompanied by an intense granulocytic response frequently leukemoid in scope. Fortunately, the vast majority of these inflammatory reactions were easily identified and caused no diagnostic problem. There remained only a relatively few cases where no obvious cause for the pronounced granulocytosis could be found. In most of these instances, the morphology of the cells, as well as their distribution in tissues, fulfilled the criteria of granulocytic leukemia.

Reticulum-cell Sarcoma.--Diagnosis of this neoplasm posed no particular problem. Characteristically, this tumor extensively involves the liver with multiple foci of variable size

composed of white tissue. These infiltrating growths were also commonly noted in the mesenteric nodes, spleen, kidneys, and less commonly in the ovaries, lungs, and thymus. The histologic pattern is one of loosely arranged large cells showing marked variation in size and configuration. Nuclear pleomorphism is pronounced and varies from relatively small ovoid nuclei to large bizarre configurations. Multinucleated cells were frequently observed in some of these tumors. Of particular interest were a few of these reticulum cell types that closely resembled classical Hodgkin's.

Aside from the four main tumor categories already noted, there remain numerous diverse neoplasms in this study. When taken as individual entities, however, the relative incidence of each of these tumor types is very small. Moreover, in many instances, only one such tumor might have been noted in the entire study but will be mentioned for the sake of completeness.

Liver.--Liver tumors are relatively common and have been the subject of much detailed research (8-12). The gross featu of these hepatomas are essentially similar, the tumors being evident as large nodular irregularities of the liver. In color and consistency, they are not unlike the adjacent normal parenchyma. On close inspection, however, the uniform lobular architecture and vascular pattern are lacking. The histology

of the hepatomas is quite uniform with variations present in cell morphology and cell-cord structure. These growths are generally well circumscribed but with no encapsulation. At the periphery there is usually compression of adjacent liver tissue, as well as obvious invasion by neoplastic cells. The hepatoma cells are lined up in an irregular cord-like fashion separated by endothelial lined sinusoids which may be entirely collapsed and devoid of blood or widely distended and congested. There are no portal areas present and no evidence of true lobule formation. The tumor cells vary from uniform cuboidal cells, which are only slightly enlarged, to the greatly enlarged polygonal cells with a great abundance of cytoplasm. This cytoplasm, in general, is somewhat more basophilic in its staining as compared to the adjacent normal liver cells. The cytoplasm is variable in its character, varying from homogeneity, to fine granularity, to marked vacuolation. Not unusual are large, spheroid, hyalin or waxy inclusions. The nuclei frequently display remarkable pleomorphism with associated hyperchromatism and chromatin clumping. In some of these hepatomas, intranuclear acidophilic inclusions are quite prominent.

Gastrointestinal Tract.--Several tumors of the gastrointestinal tract were noted (10,13,14). The majority of these were of gastric origin. Squamous cell carcinoma arising from the forestomach accounted for four of the tumors. Infiltration

was extensive with involvement of the liver and pancreas. Two mucoid carcinomas (mucin-secreting adenocarcinomas) and one adenomatous polyp were also readily identified. No distant metastases were noted. In addition, there were two anaplastic small celled neoplasms and an unusual mixed tumor with a sarcomatous stroma containing neoplastic glandular elements and foci of osseous deposition. Throughout the intestinal tract only two lesions were noted. Both consisted of aggregates of atypical glands within the muscularis and could not definitely be classified as true neoplasms on morphology or location. One large adenocarcinoma was found in the anal region, but the site of origin could not be identified.

Adrenals.--Of the endocrine system, not including the gonads, only the adrenals were identified as being the site of tumor formation. No specific search was made for neoplasia of the hypophysis or thyroid-parathyroid regions. Tumors of the adrenal were predominantly cortical in origin. These all appeared to be benign adenomas composed of uniform cuboidal cells arranged in irregular cords and differing only slightly from the normal cortex. In general, the adenoma cells were slightly larger and the cytoplasm somewhat paler and lacking fine vacuolation or granularity. One adrenal tumor had cellular characteristics indicating possible medullary origin.

These cells were large and elongated, often spindle-shaped, and some showed granularity of the cytoplasm. An indistinct cord-like configuration was the predominant pattern, although some of the cells were clumped together in small nests. The nuclei revealed only slight pleomorphism, and mitoses were rare.

Kidneys.--The finding of renal neoplasia was restricted to six tubular adenomas. These were all small in size and located at the periphery of otherwise normal cortical tissue. No encapsulation was present; however, the tumor border was well delineated. The histologic pattern was one of atypical tubule formation. The lumens varied from almost obscure slits to widely distended cystic spaces containing amorphous acidophilic globules and cell debris. The cells are high cuboidal and not infrequently heaped up into papillary projections within the lumens. The cytoplasm is acidophilic and generally finely granular. The nuclei are relatively small, uniformly rounded, and usually situated parabasally. One of the adenomas had an area of cellular and nuclear pleomorphism. No mitoses were noted, and this is considered a degenerative rather than malignant alteration.

Epidermal Tumors.--The occurrence of tumors arising from the epidermis was extremely rare, both in the irradiated mice and in the controls. One epidermoid carcinoma was present

in the external ear region and displayed classical histology with broad tongues of epidermoid cells invading into the dermis; epithelial pearls were readily identified. The oral mucosa contributed three squamous cell carcinomas which had widely infiltrated the adjacent structures, including buccal wall and mandible.

Mesenchymal Tumors.--Many neoplasms in this category were found; however, most have not been definitely classified as yet. In general, the full scope of mesenchymal tumors is noted. The gross and microscopic characteristics of these tumors parallel very closely those noted in man. Both benign and malignant varieites are well represented. The sarcomas predominated, probably due to their rapid growth and relatively large size at autopsy. Among the mesenchymal tumors present in this series are fibroma, fibrosarcoma, neuroma, neurogenic sarcoma, osteoma, osteosarcoma, chondrosarcoma, and angioma.

Discussion

Basically, the study thus far completed constitutes a survey of pertinent literature regarding neoplasms identified in laboratory mice. From these accumulated data, a workable classification has been compiled and the tumors noted within this series applied thereto. In general, there has been no

significant histologic deviation from the categories herein described. As might be expected in any autopsy study of this magnitude, there are several neoplasms which fail to fulfill the histologic criteria of any of the above classes. These tumors must, of necessity, be more fully evaluated. The completion of this study awaits the final correlation as to tumor incidence as a function of radiation.

References

(1) H. G. Grady and H. L. Stewart, Am. J. Path. 16, 417 (1940).

(2) H. A. Horn, C. C. Congdon, A. B. Eschenbrenner, H. B. Andervont, and H. L. Stewart, J. Nat. Cancer Inst. 12, 1297 (1952).

(3) S. W. Lippincott, J. E. Edwards, H. G. Grady, and H. L. Stewart, J. Nat. Cancer Inst. 3, 199 (1942).

(4) H. L. Stewart, Pulmonary Tumors in Mice, Chap. 2, In: Physiopathology of Cancer, Hoeber-Harper, N. Y., 1959.

(5) F. K. Mostofi and C. D. Larsen, J. Nat. Cancer Inst. 11, 1187 (1951).

(6) T. B. Dunn, Morphology of Mammary Tumors in Mice, Chap. 3, In: Physiopathology of Cancer, Hoeber-Harper, N. Y., 1959.

(7) T. B. Dunn, J. Nat. Cancer Inst. 14, 1281 (1954).

(8) H. B. Andervont and T. B. Dunn, J. Nat. Cancer Inst. 15, (1955).

(9) H. B. Andervont and T. B. Dunn, J. Nat. Cancer Inst. 13, 455 (1952).

(10) A. M. Cloudman, Spontaneous Neoplasms in Mice, Chap. 4, In: Biology of the Laboratory Mouse, Dover Pub. Inc., N. Y., 1941.

(11) H. I. Firminger, J. Nat. Cancer Inst. 15, 1427 (1955)

(12) H. L. Stewart and K. C. Snell, The Histopathology of Experimental Tumors of the Liver of the Rat, Chap. 4, In: Physiopathology of Cancer, Hoeber-Harper, N. Y., 1959.

(13) E. A. Saxen and H. L. Stewart, J. Nat. Cancer Inst. 1 657 (1952).

(14) H. L. Stewart and E. Lorenz, J. Nat. Cancer Inst. 3, 175 (1942).

Giant Cell Formation in HeLa Cells as a Function of Co^{60} Gamma and X Irradiation (D. C. White and P. C. Sanders)

Introduction

It has been shown that both Co^{60} gamma rays and X rays produce giant cell forms in a variety of established cell strains. This biological phenomenon, although typical of cell strains, has also been reported in early passages of primary explant cells of several types. The mechanism of giant cell formation is not fully understood, but further studies of these altered cells have shown them to be incapable of cell division and, therefore, colony formation. The purpose of this study was to correlate the per cent of giant cells formed in HeLa cells with the total dose of radiation.

Materials and Methods

All exposures were made in Rose chambers (1) inoculated with S-3 HeLa cells using approximately 5,000 cells per chamber. These cells were grown in Eagles basic medium plus 10 per cent horse serum for 48 hours preceding exposure to Co^{60} or X rays. Cobalt exposures were carried out at the source using a configuration that permitted the simultaneous subjection of 60 chambers to a uniform dose rate of 5 r/min.

In order to keep the results of X irradiation comparable, such distance was employed so that the dose rate of these exposures also was 5 r/min.

In a further effort to make the results as statistically valid as possible, no less than 10 chambers were used at each predetermined total dose. On each "run," the dose groups were overlapped; the repetitive groups served as a check on the validity of the newly added dose groups.

Employing these methods, the following dose groups were examined: 0, 75, 150, 300, 450, 600, 750, 900, 1000, and 1200 r. A total of 149 Co^{60} chambers and 40 X-ray chambers were exposed. Following irradiation, the chamber cultures were incubated for an additional 5 days with medium changes immediately following irradiation and again on the second post-irradiation day. On the fifth day, the chambers were dismantled. The coverglass cultures were washed with phosphate buffered saline, fixed with absolute methyl alcohol, and stained with the Jacobson's method (May-Grünwald-Giemsa).

For the determination of giant cell percentages, all cells were included which had at least twice the total area of the standard HeLa cell. With few exceptions, the nuclei of these giant cells exhibited morphologic alterations including macronuclei, multiple nuclei, bizarre configurations, and atypical chromatin formation. Not included in the

giant cell count were those cells showing only nuclear distortion or fragmentation. The number of these latter cells remained essentially constant at all dose levels and did not appear to be an integral part of giant cell formation. For each coverglass preparation, a total of 1,000 cells were counted in random microscopic fields using a 10x objective and 15x oculars.

Results

The irradiation of HeLa cells employing the method described gave a readily reproducible curve of giant cell production as a function of total radiation dose. Figure 1 shows the dose response relationship.

Evaluation of the control chambers revealed a consistent 1 per cent giant cell formation, which is in agreement with observations of others, who have undertaken similar studies using the HeLa cell. All chamber counts at the prescribed dose levels are in close agreement and do not represent the average value of widely variable determinations. It is of interest to note that the giant cell values for X-ray exposures are consistently somewhat higher than those obtained with Co^{60} gamma irradiation.

Although the results thus far indicate a definite correlation between giant cell formation and total dose of radiation, it is apparent that much additional investigation must be

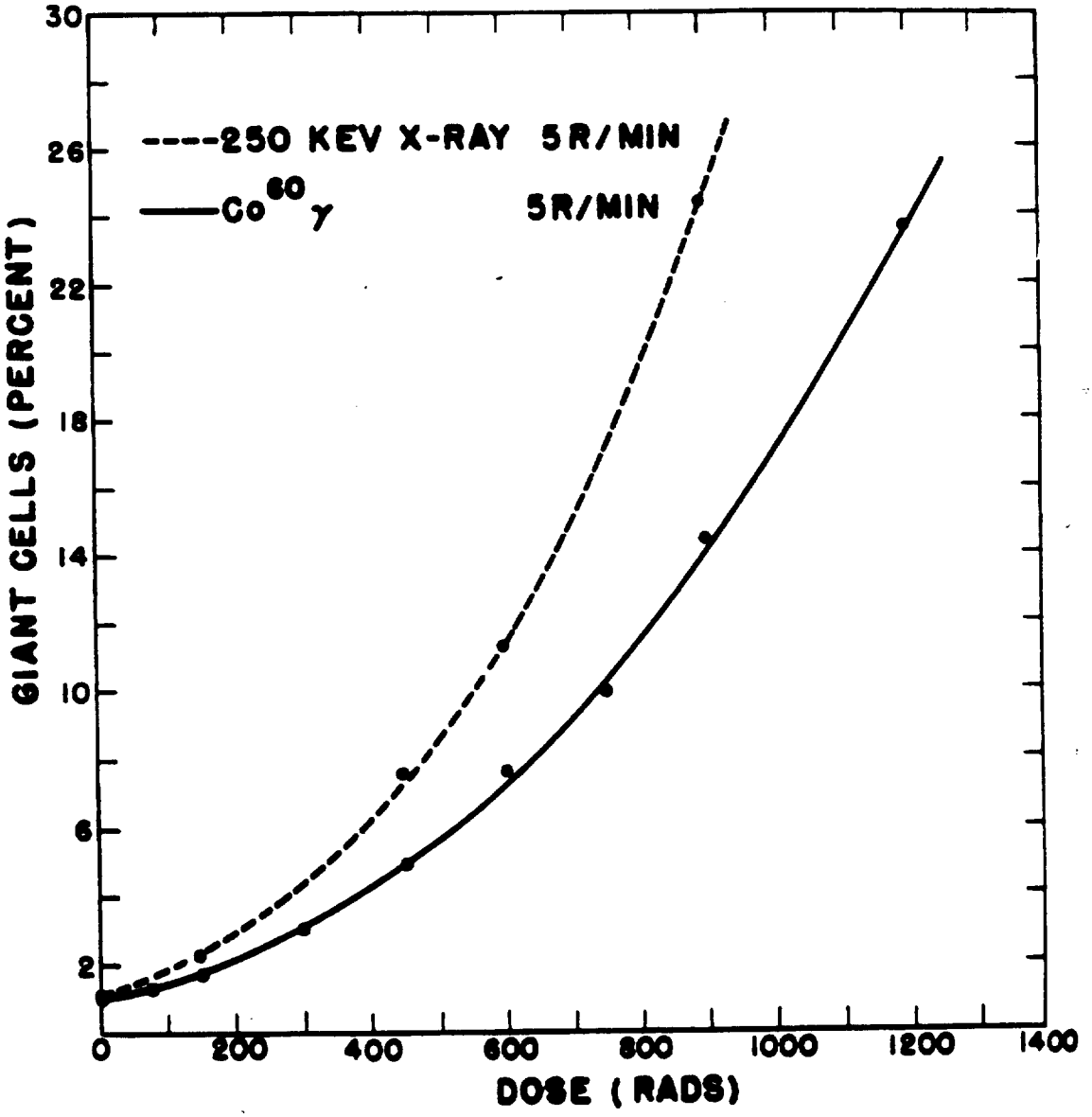

Fig. 1. HeLa giant cell production as a function of radiation dose.

carried out in order to establish a satisfactory base line for further studies pertaining to the relative biological effectiveness (RBE) of other types of radiation. Further studies should include the effect of dose rate, evaluation of total cell survival, and determination of colony plating efficiency.

It seemed reasonable to assume that under rigidly controlled experimental conditions this biological response to ionizing radiation might prove to be an important adjunct to the evaluation of the relative effects on HeLa cells of a variety of radiations of different types and energies.

Reference

(1) G. G. Rose, Texas Rep. Biol. a. Med. 12, 1074 (1954).

Toxicity, Metabolism, and Tissue Distribution of C^{14}-labeled Triethylene Thiophosphoramide (thio-TEPA) in Rats (I. U. Boone and D. L. Williams)

Introduction

The nitrogen mustard and triethylene melamine content of normal and tumor tissue after intravenous and intra-arterial injection in rats has been previously reported from this Laboratory (Cancer Res., 17, 1120-1126, 1957). A similar study has been completed with C^{14}-labeled triethylene thiophosphoramide (C^{14}-thio-TEPA) in normal rats.

Methods and Results

The relative toxic effect of thio-TEPA administered by intravenous and intra-arterial injections was determined for a series of graded doses. The doses were 8, 9, 10, 11, and 12 mg/kg body weight. Six to 12 rats were injected at each dose level.

The concentrations of C^{14}-labeled thio-TEPA and metabolites were measured in the various tissues following intravenous and intra-arterial injection of 9 mg/kg body weight of the drug. Since alkylating agents of this type react rapidly in the body, the animals were sacrificed 5 minutes after injection and the various tissues removed for analysis.

In addition to the tissue analysis for C^{14} content, the metabolism of the drug was studied over a 24-hour period. With the use of standard glass metabolism cages, as previously used and described in this Laboratory, the excretion pattern in urine, feces, and expired air (CO_2 content) was studied. The metabolism of the C^{14}-thio-TEPA was further investigated through the use of urinary chromatograms. All samples were counted by the liquid scintillation method. All counting was done in a Los Alamos Model 540 coincidence system counter.

An attempt was made to correlate histological damage with the concentration of the drug in the tissues and the route of injection. Normal rats were given injections intra-arterially and intravenously of 9 mg/kg body weight of thio-TEPA and sacrificed for histological study at intervals of 1, 3, 7, and 14 days. A summary of the results follows.

1. There was no significant difference in the survival time of normal rats when treated with thio-TEPA by either of the two injection routes at any of the dose levels treated. The LD_{50} dose by either route was 10 mg/kg body weight.

2. The distribution of C^{14} activity from labeled thio-TEPA in tissues of normal rats given injections intravenously and intra-arterially is summarized in Table 1. Of the tissues investigated, only the testes, liver, and blood cells differed significantly in concentration between the two routes of

TABLE 1. Tissue Distribution of C^{14} Activity 5 Minutes after Intravenous and Intra-arterial Administration of C^{14}-thio-TEPA to Rats

Sample	Intra-arterial*	Intravenous*
Thymus	9.0	9.4
Lungs	12.3	10.2
Spleen	9.0	6.8
Kidneys	17.9	10.2
Gastrointestinal Tract	3.9	4.8
Brain	8.8	7.2
Muscle	9.7	7.9
Heart	10.4	10.6
Testes	4.7	7.8**
Liver	9.4	12.6**
Blood Cells	8.8	5.9**
Plasma	13.9	11.6

*Results expressed as μg equivalents/gm of tissue.

**Values differ significantly ($P \geq 0.05$) for the two routes of administration.

injection. These results more closely resembled those previously obtained with triethylene melamine (TEM) rather than nitrogen mustard (HN_2). Carbon^{14} activity following the injection of thio-TEPA, regardless of the route of injection, was more evenly distributed throughout all tissues than either TEM or HN_2.

3. Table 2 summarizes the 24-hour excretion data. Between 87 and 98 per cent of the drug is excreted in the urine in the first 8 hours following injection.

4. Chromatograms of urine samples indicate that the majority of the injected drug is excreted as thio-TEPA. The R_f value of thio-TEPA with either n-butanol saturated with water or 80 per cent ethanol as solvent systems was $\sim$0.86. Two or three other unidentified metabolites were present in lesser amounts than the thio-TEPA.

5. Histopathological findings following the administration of thio-TEPA were not influenced by the route of administration or tissue concentrations of the drug and its metabolites.

TABLE 2. Carbon^{14} Activity Excreted in 24 Hours following Administration of C^{14}-thio-TEPA to Rats

Urine (per cent)	Feces (per cent)	Expired CO_2 (per cent)	Total (per cent)
95.9	0.98	2.14	99.02

Metabolism of C^{14}-Isoniazid in Humans (I. U. Boone, L. M. Conklin, G. Trafton, and R. Des Prez*)

Introduction

The hydrazide of isonicotinic acid (isoniazid) is one of the more promising drugs for the chemotherapy of tuberculosis. Labeling of this important therapeutic agent with C^{14} by Murray and Langham (1) has afforded an additional means of studying its metabolism in animals and man.

The present study was a joint project between the University of California's Los Alamos Scientific Laboratory and *U. S. Public Health Service Hospital at Fort Defiance, Arizona, and Department of Public Health and Preventive Medicine, Cornell University, New York Hospital Medical Center, New York City, the purpose of which was to study the metabolism of C^{14}-isoniazid in tubercular and nontubercular patients.

Methods and Results

The metabolism of isoniazid was studied in tubercular and nontubercular patients following intramuscular administration of approximately 300 μc of C^{14}-isoniazid. Carbon^{14} activity in blood and urine was followed for 24 hours. Urine C^{14} content was determined over an additional 48-hour period. Carbon^{14} levels in the spinal fluid were determined 2 hours after drug administration. Chromatographic analysis of the

urine samples was performed, and chemical separation of the major excretory products was carried out and the amount of radioactivity determined.

In 2 patients, C^{14}-isoniazid determinations were repeated following daily administration of 300·mg of pyridoxine for 3 weeks. In 4 patients, the C^{14}-isoniazid was repeated following 3 to 4 weeks of daily para-aminosalycilic acid (PAS) administration. Findings have indicated the following:

1. Seventy-five to 95 per cent of the dose was excreted in the urine in 24 hours.

2. Levels in the spinal fluid 2 hours after drug administration were found to be approximately one-fifth the serum level at this time.

3. Chromatographic separation of urinary metabolites of the C^{14}-isoniazid demonstrated at least three major bands. By means of R_f values of known samples and infrared spectrometry, the acetyl isoniazid and isonicotinic acid were unequivocally identified. Only very small amounts of unchanged C^{14}-isoniazid were present. Although the degree of acetylation varied widely between individuals, it appeared to be a constant biological characteristic of single individuals. Some subjects excreted as much as 60 per cent of the isoniazid as the acetyl derivative, while others as little

as 20 per cent as the acetyl isoniazid.

4. Very little or no change in the pattern of urinary metabolites could be induced by oral administration of either pyridoxine or PAS.

Reference

(1) A. Murray III and W. H. Langham, J. Am. Chem. Soc. 74, 6289 (1952).

Preparation of a Linear Alpha Source (T. T. Trujillo, J. D. Perrings, and J. M. Wellnitz)

Introduction

In connection with studies of effects of heavy primary cosmic rays, it may be helpful to have a linear alpha source for exposing tissue cells either in a mass or in single layers. This source should produce uniform radiation and allow means to establish dose received by the cells. One possible method for preparing such a source is the electroplating of a known amount of plutonium on a very thin wire that could be introduced into the cell culture. This investigation was conducted in an attempt to obtain such a source that would meet some of the desired specifications.

Methods and Results

A solution of Pu^{239} in 4N HNO_3 was used; however, it was necessary that the plutonium be in the +6 state and in a 1N KOH solution for the electroplating process. The method for preparing this basic solution is described in a previous Los Alamos Report (1). A solution containing 1,200,000 d/m/ml was prepared by the above method. The apparatus for the electroplating process is shown in Fig. 1. Platinum wire 0.003 in. thick, sealed into a hypodermic needle, was used as the cathode for electrodeposition of the plutonyl ion. The cathode was

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Fig. 1. Electrodeposition apparatus showing electrodes and plutonium container in disposable Lucite box.

passed through another hypodermic needle of larger gage into the Lucite box used to confine the plating operation in event of spattering or an accidental spill of the solution. A vial containing approximately 2 ml of plutonyl KOH solution was raised to submerge the anode (platinum wire coil) and cathode. The current was supplied by three 1-1/2 volt dry cells. Two platinum wires approximately 2 cm in length were electroplated for periods of 5 and 30 minutes, respectively, and counted in a gas flow proportional counter.

The two wires electroplated for 5 and 30 minutes counted 24,000 and 136,000 d/m, respectively (assuming a 50 per cent counting efficiency). Radioautographs of these sources were subsequently made to observe distribution of plutonium on the wire. Figure 2 shows radioautograph of the second source prepared by embedding in paraffin and placing against a nuclear track photographic emulsion. The wire was embedded in the paraffin to approximately half its diameter to prevent exposure of the emulsion from scattered alpha particles.

From this preliminary study, it is evident that electrodeposition of the plutonyl ion can be accomplished on thin platinum wire, and the plutonium appears to adhere tightly and to be deposited uniformly (Fig. 2). The source can be counted in a special counter and an estimation of total activity observed for dose estimation purposes. Preparation of the sources from

Fig. 2. Autoradiograph of plutonium deposited on 0.003 in. diameter platinum wire, 130,000 d/m (270X).

Pu^{238} would increase the deposited activity and the dose rate by a factor of about 270.

Reference

(1) J. McClelland, Analytical Procedures of the Industrial Hygiene Group. Los Alamos Scientific Laboratory Report LA-1858 (2nd edition), 1958.

RADIOBIOLOGY SECTION PUBLICATIONS

(1) E. R. Ballinger and P. S. Harris, Field Study of the $AgPO_3$ Glass Personnel Dosimeter (U. S. Navy DT-60). Los Alamos Scientific Laboratory Report LA-2298 (1959).

(2) I. U. Boone, Effects of Partial-body and Whole-body X-irradiation on Life Span and Tumor Incidence of CF_1 Mice, Abstract No. 12, Rad. Res. 11, 434 (1959).

(3) P. S. Harris, E. F. Montoya, and W. H. Schweitzer, Dose and Flux Measurements on Godiva Radiation Effects Experiments. Los Alamos Scientific Laboratory Report LA-2355 (1959).

(4) P. S. Harris, The Acute Effects of Nuclear Radiations from Nuclear Weapons. In: Hearings before the Special Subcommittee on Radiation of the Joint Committee on Atomic Energy, Congress of the United States, Eighty-Sixth Congress, First Session on Biological and Environmental Effects of Nuclear War (June 22-26, 1959), pp. 267-273.

(5) P. S. Harris, Summary of the Acute Effects of Nuclear Radiations from Nuclear Weapons. In: Hearings before the Special Subcommittee on Radiation of the Joint Committee on Atomic Energy, Congress of the United State Eighty-Sixth Congress, First Session on Biological and

Environmental Effects of Nuclear War (June 22-26, 1959), Summary Reports, pp. 37-38.

(6) J. A. Sayeg, E. R. Ballinger, and P. S. Harris, Dosimetry for the Godiva II Critical Assembly. Neutron Flux and Tissue Dose Measurements. Los Alamos Scientific Laboratory Report LA-2310 (1959).

(7) J. F. Spalding, V. G. Strang, and J. A. Sayeg, Relative Biological Effect of Fission Neutrons with the Broad Bean Root (Vicia faba) as a Test System, Rad. Res. 10, 176 (1959).

(8) J. F. Spalding, S. B. Hawkins, and V. G. Strang, The Ability of Extrinsic Gonadotropin (FSH) to Function when Administered after Sublethal and Supralethal X-ray Exposures, Rad. Res. 11, 67 (1959).

(9) T. H. Allen, B. E. Welch, T. T. Trujillo, and J. E. Roberts, Fat, Water and Tissue Solids of the Whole Body Less Its Bone Mineral, J. Appl. Physiol. 14, 1009 (1959).

(10) F. C. V. Worman and P. S. Harris, A Graphite-CO_2 Ionization Chamber Instrument for Gamma Ray Dose Rate Measurements with a Six Decade Range, Fast Response and Remote Recording. Los Alamos Scientific Laboratory Report LA-2361 (1959).

MANUSCRIPTS SUBMITTED AND ACCEPTED

(1) I. U. Boone, Incidence of Tumors in Animals Exposed to Whole-body Radiations, to be published in ORO-SP-127. Proceedings of the Symposium on the Delayed Effects of Whole-body Radiation, held in Washington, D. C. on October 29, 1959.

(2) J. F. Spalding, V. G. Strang, and F. C. V. Worman, Effect of Graded Acute Exposures of Gamma Rays or Fission Neutrons on Subsequent Survival in Protracted Gamma-ray Exposures, Rad. Res.

CHAPTER 6

RADIOPATHOLOGY SECTION

Clinical Applications of Whole-Body Scintillometry. I. Retention of Orally Administered Iron (C. C. Lushbaugh and D. B. Hale)

Introduction

Iron was one of the first metals to be recognized as necessary for normal human metabolism. Hippocrates is known to have administered a broth from iron filings steeped in wine to people who were pale and appeared to have "thin" blood. Since then, oral iron medication has been the accepted treatment for anemic states. When such oral treatment is not effective, parenteral iron has been given empirically to overcome the apparent inability of the intestine to absorb iron. Numerous postulates have been made to explain this apparent blockage of mucosal absorption, but again today there is a serious question as to whether such a mucosal blockage exists.

The human body has no means of excreting absorbed iron. Hypochromic anemia, therefore, can result only from iron loss through hemorrhage or from failure of dietary iron to be absorbed.

There is today no simple means of determining whether any particular patient will absorb ingested iron. The methods available for such determinations rely upon quantitative analysis of feces for either stable iron or Fe^{59} used as a tracer (1). While use of this isotope has simplified the laborious iron analyses, it has not solved the difficult problem of making quantitative fecal collections in fastidious patients. As a result, iron balance studies are still difficult to do and are performed only in research and not in medical practice.

The whole-body counter may be used to determine the fate and absorption of orally administered iron by measuring the per cent of the dose which is retained permanently in the body. These measurements can be made directly and easily with the large liquid scintillation counter, since Fe^{59} has a strong gamma ray for which the machine has a high efficiency.

Methods

Oral uptake and retention of Fe^{59} have been studied in 66 persons. After a normal gamma ray measurement, each individual ingested 0.5 to 0.7 μc Fe^{59} (specific activity 3 μc/mg) as ferrous citrate in 1 per cent ascorbic acid solution to maintain the iron in the reduced state. The dose was administered from a plastic spoon and followed by ingestion of 100 to 200 ml of water or carbonated beverage. The patient was counted immediately to determine the counting rate as modified by the

patient's mass. In this way, geometric and mass absorption problems resulting from the wide variation in size of subjects were minimized.

Fecal samples from the first 10 patients were collected quantitatively in plastic containers and counted to obtain a "balance" between ingested and retained plus excreted iron. When corrected for decay, these data revealed that even in constipated patients the retained activity was essentially constant after 1 week (Fig. 1). It was possible, therefore, to set up a routine method of analysis whereby the patient was counted for 200 seconds immediately after taking the radioactive iron and again only on the seventh day. With this method, it was then possible to minimize the amount of time required for the determination. Both counts were related to a sealed standard containing an amount of iron equivalent to that ingested by the patient to correct for radioactive decay. An additional 56 patients were studied using the latter method. Clinically, the 66 subjects were classified as follows.

Thirteen were normal or had received previous parenteral or oral iron medication. At the time of this study, they all had hemoglobin values that were considered normal.

Nineteen of the subjects were considered to be anemias secondary to acute infections (all had had previously normal hemoglobin determinations and were found, during an acute infection, to have unexpected anemia).

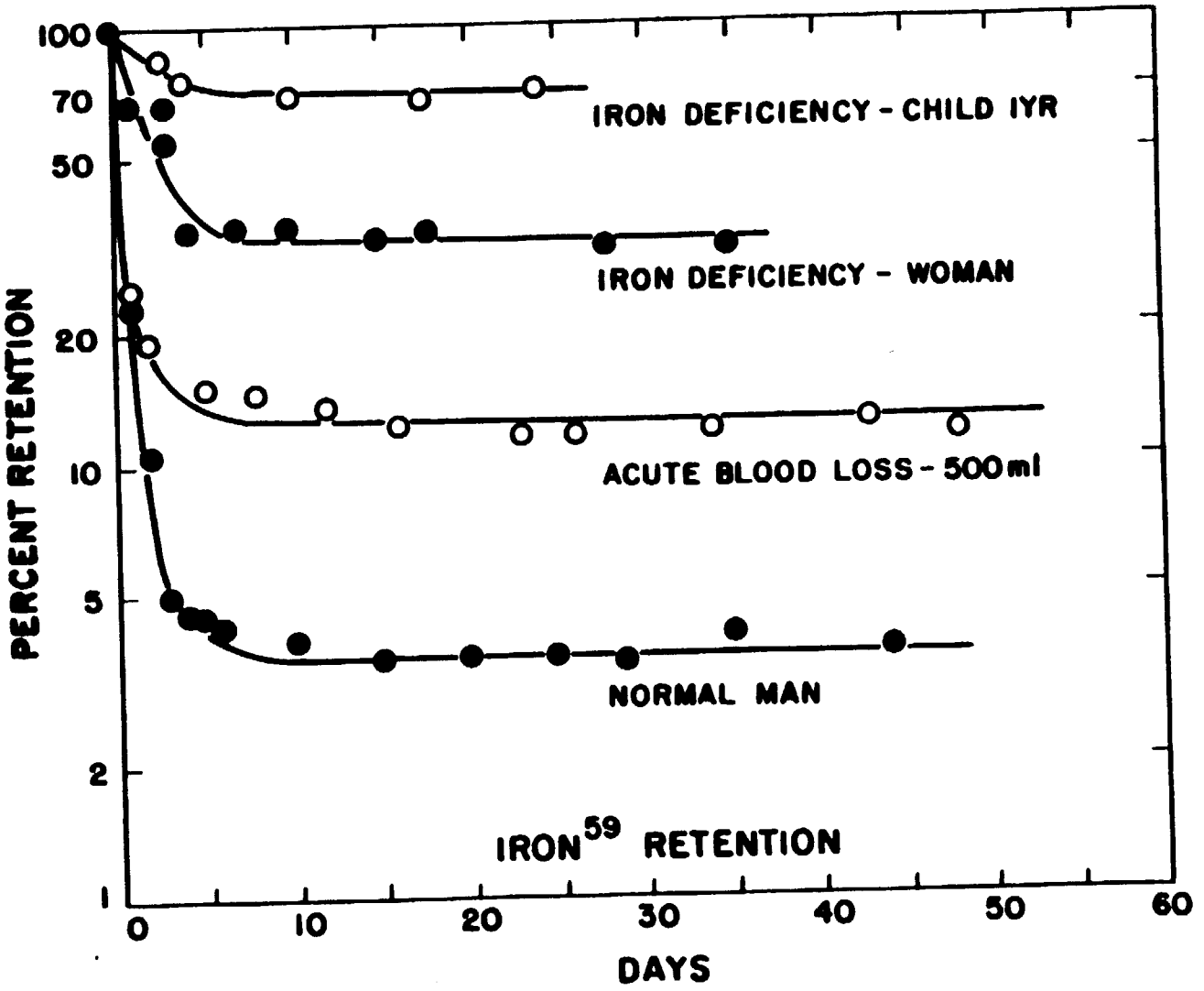

Fig. 1. Retention of orally administered Fe^{59}-citrate in a normal man, in a normal man following acute blood loss, in an anemic woman following chronic menstrual hemorrhage, and in an anemic child due to severe dietary iron deficiency.

Three patients had anemia secondary to various leukemias, and 3 have varieties of polycythemia (1 polycythemia rubra vera, 1 polycythemia secondary to pulmonary disease in which infection was a component, and 1 familial erythrocytosis).

Twenty-eight patients were considered as typical hypochromic anemias. Four of these were Spanish-American children, who had been fed milk by breast or bottle as their only source of nutriment for over one year and were compulsive dirt eaters, and one (G) was a 7-months pregnant woman.

Results and Discussion

Figure 1 shows Fe^{59} retention curves typical of 4 different conditions. One is for a normal healthy man, and another for the same man after 500 ml of blood had been withdrawn by venasection. The third is for a moderately hypochromic anemic adult woman, and the fourth is for a severely anemic dirt-eating child with a 4-g hemoglobin. All curves appear to be a composite of two exponential lines, one representing fecal transit time of the unabsorbed intraintestinal iron dose, and the other (with no appreciable slope) representing the retention of the Fe^{59} absorbed from the intestinal tract. Contribution of the first exponential to the over-all excretion curve becomes insignificant at

about the fifth day, thus allowing use of the seventh day as the time for the second (and last) count as a measure of iron absorption.

Iron^{59} absorption is contrasted with whole blood hemoglobin in Fig. 2. A rough correlation exists between iron absorption as measured by this method and the hemoglobin values of the hypochromic patients (H) without infection. Patients recovering or still suffering from various acute or chronic infections (I) do not appear to show any enhancement of iron absorption above that seen in the normal patients (N). The anemic leukemic patients (L) likewise did not show increased iron absorbability. Polycythemic patients (P) stand out remarkably because of their high hemoglobin and increased iron absorption. The one patient (PI) with polycythemia secondary to chronic bronchiectasia and degenerative emphysema does not show this phenomenon. If the explanation for this example of increased absorption in polycythemia rubra vera is etiologic, then rate of intestinal absorption of iron would be a much simpler method of differentiating it from secondary polycythemia than present methods.

The only pregnant woman studied (G) had a 11.5-g hemoglobin and absorbed 99 per cent of the Fe^{59} tracer dose. At parturition two months later, the placenta was preserved so

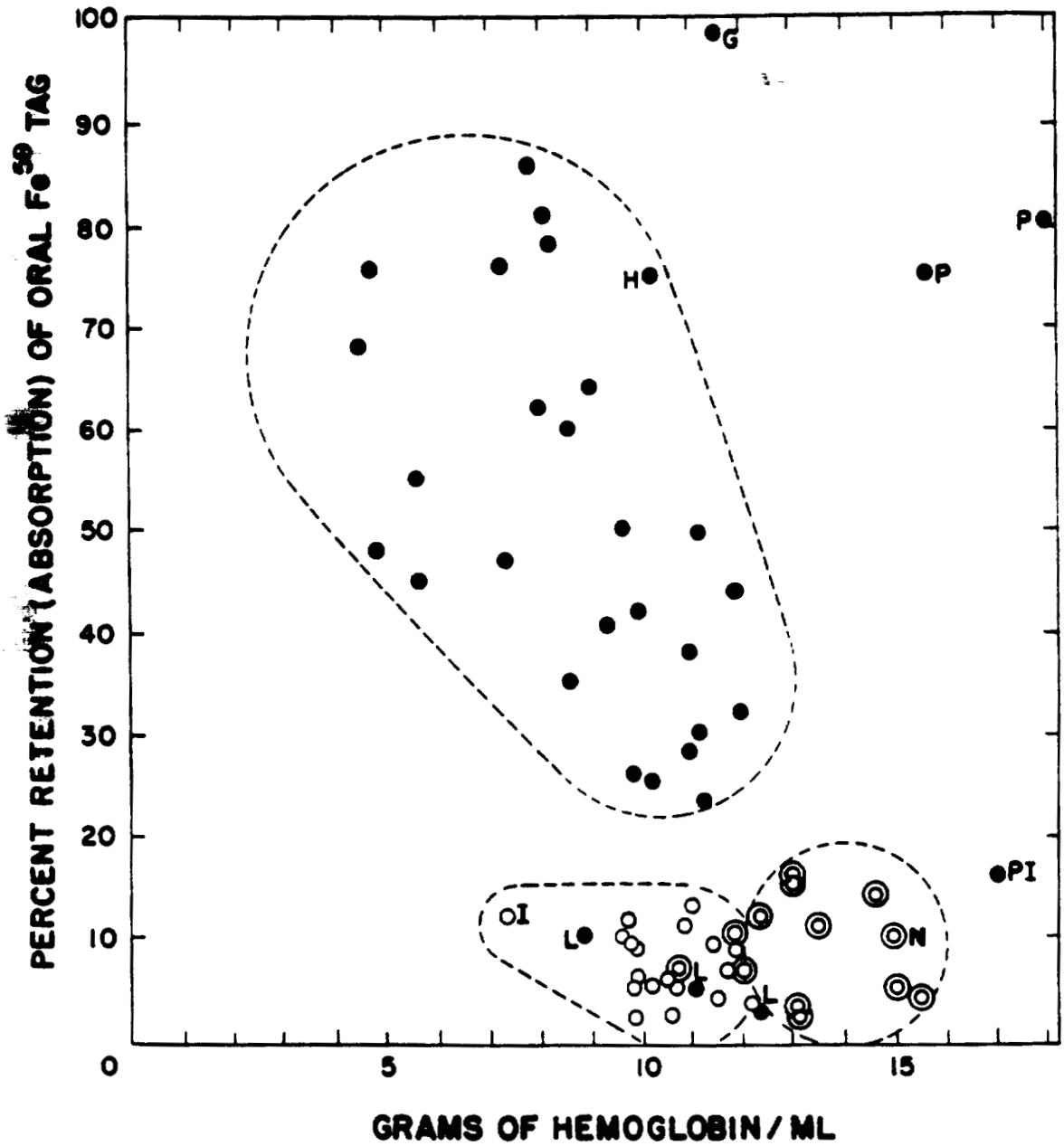

Fig. 2. Absorption and retention of orally administered Fe^{59} in relation to the peripheral blood hemoglobin of patients. (N) = so-called normals; (H) = hypochromic anemias; (P) = polycythemias; (I) = post infectious anemias; (L) = leukemias; (G) = a 7-months pregnant woman; and (PI) = a polycythemic man with severe pulmonary disease.

that it, the mother, and the new born child could be radioassayed on the day the child was discharged from the hospital. At that time the mother was found to have retained 48 per cent, the placenta 10 per cent, and the child 23 per cent of the original dose. The mother had hemorrhaged heavily requiring a post-partum transfusion, probably explaining the deficit of 18 per cent. It would seem, therefore, that this 18 per cent should be added to the maternal percentage, making her net absorption at the time of administration of the dose 66 rather than 99 per cent.

The poor correlation between hemoglobin and iron absorption led to an attempt to relate absorption to serum iron content. Figure 3 shows the correlation in the first 12 patients for which serum iron levels were obtained at the time of oral administration of the tracer. Additional cases are being obtained with serum iron and serum iron binding capacities in order to test the apparent exponential relationship which Fig. 3 seems to indicate. The patient with less than 2 per cent retention and a serum iron of only 27 µg had chronic pyelonephritis, chronic menstrual blood loss, and poor nutrition. This case illustrates that intestinal absorption of iron is not necessarily correlated with the serum iron level or bodily need for iron as postulated by Granick (2). Whether this failure to absorb iron is due

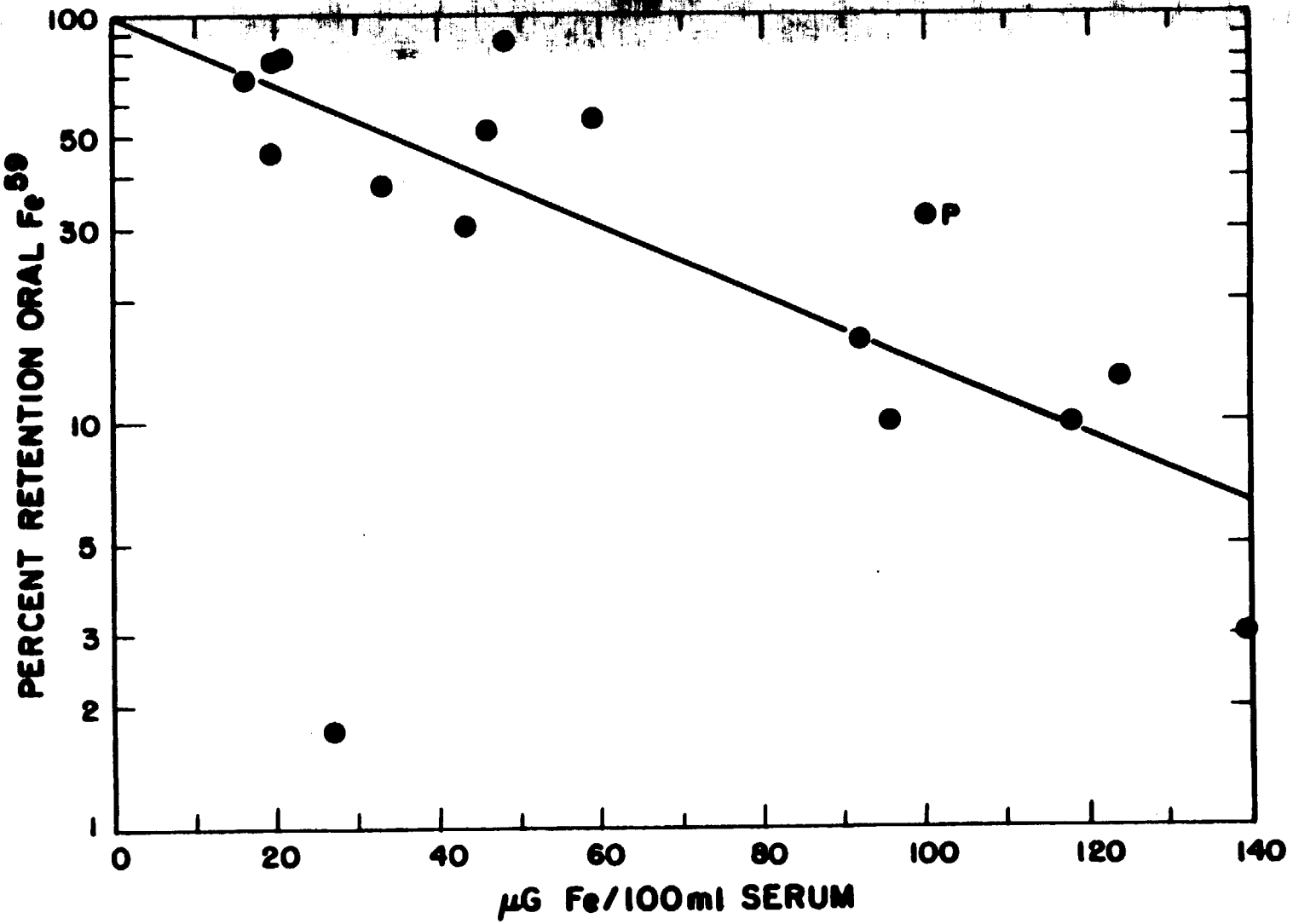

Fig. 3. Relationship between oral iron absorption and serum iron content.

to a true "mucosal blockade" in this case cannot be determined by this method. The method shows, however, that oral iron medication would be useless in such a case of hypoferremia. Since serum iron was not determined, the apparent failure of Fe^{59} absorption in the infectious cases (I) and in the leukemia patients (L) cannot be discussed from the point of view of apparent iron need. However, upon recovery from their infection, the majority of cases of uncomplicated acute respiratory infection showed a return to normal hemoglobin levels without iron therapy. In those infected cases given oral or parenteral iron therapy, hemoglobin recovery did not seem to be hastened, apparently since their iron stores were not depleted. These observations seemed particularly true in cases of acute and subacute hepatitis.

Conclusions

The whole-body counting technique for determining per cent retention of an orally administered tracer dose of Fe^{59} appears to be a facile means of measuring absorption of orally administered iron. The preliminary study reported here has revealed that there are many facts about intestinal iron absorption in various diseases and normal physiologic states which warrant further investigation. The apparent lack of iron absorption in the anemias of the three leukemia

and in acute infections is not understandable in the light of present knowledge. The remarkably high iron absorption by the pregnant woman and the large proportion of the oral dose found subsequently in the placenta and infant emphasize the need for more data on oral iron retention in pregnancy. The increased sensitivity of the new human counter now under construction will provide accurate measurements of Fe^{59} uptake with doses of less than 0.1 μc. This will permit such studies to be made more safely and more frequently in young persons and in early pregnancies.

References

(1) H. W. Josephs, Blood 13, 1 (1958).

(2) S. Granick, Physiol. Rev. 31, 489 (1951).

Clinical Applications of Whole Body Scintillometry. II. A Comparison of Three Different Methods of Determining Retention and Thyroid Uptake of Orally Administered I^{131}

(C. C. Lushbaugh and P. S. New)

Introduction

Although studies of thyroid uptake of I^{131} constitute the largest single use of radioisotopes in clinical medicine, these studies are commonly inaccurate and difficult to interpret. In spite of recent attempts to standardize techniques (1), only the extremes of thyroid dysfunction are universally detectable or interpretable because of inherent errors in the methods of measurement. Overcoming these sources of error, which are largely geometric, would appear to be possible by measurement of the entire body rather than the gland itself. Positioning the whole body would not be as difficult as positioning the neck, and the initial whole body count could be used as the 100 per cent dose, thereby making each patient his own standard and eliminating the error of self-absorption.

Two instruments for determination of radioactivity in the whole body are presently available. The one most commonly

known locally as the "Los Alamos Human Counter" and designated Humco I.

Comparisons of whole body counting with conventional local counting of the gland itself (1), as a method for determination of thyroid I^{131} retention, were followed in 17 patients chosen at random using a 9 x 6 in. sodium iodide crystal, the whole body liquid scintillation counter, and a 3 x 3 in. collimated sodium iodide crystal placed over the gland.

Methods and Materials

Both the 9 x 6 in. sodium iodide crystal scintillometer and the 4π liquid scintillometer have been described previously (2-4). Both the 9 x 6 in. and the 3 x 3 in. crystals were mounted on an adjustable trolley and enclosed in a steel room 10 x 10 x 7 feet in size in order to achieve a low background. In these experiments, only a single channel analyzer was used with the crystals. The lower gate discriminator was set at 260 kev so that no pulses were recorded which fell below that energy, thus eliminating most of the low energy back scatter. The liquid scintillation counter, being unable to detect soft gamma rays below 500 kev, recorded only the 640 kev emission of I^{131}. While these emissions represented only 10 per cent of the total gamma emission

from I^{131}, the efficiency of the liquid scintillometer was high enough to detect changes of the same order as detected by the sodium iodide crystals.

Table 1 shows the clinical classification of the 17 patients and the number of determinations made. They were given 1.5 to 3.0 μc I^{131} as carrier-free sodium iodide by mouth, and each group of 3 patients was related to a "neck" phantom which contained the same amount of I^{131} administered to the patient. This phantom, modeled after Brucer (1), consisted of a Lucite cylinder 12.5 cm in diameter and 18 cm high with a well inside large enough to accommodate a plastic test tube containing the I^{131} in a volume of 25 ml. The anterior surface of the tube was 1.5 cm from the surface of the cylinder. This phantom standard was counted each time the patient was radioassayed in the scintillation counter or by the 3 x 3 in. collimated crystal. It was not used in conjunction with the 9 x 6 in. crystal, as the stability of the background here allowed for mathematical correction for isotopic decay after the initial radioassay of the patient. The patients were assayed as soon as possible after administration of the dose (5 to 30 minutes) and at daily intervals for about 14 days. The 9 x 6 in. crystal surface was 90 cm from the thyroid cartilage, the lumen of the stomach, and the surface of the thighs (Fig. 1

TABLE 1. Clinical Classification of Patients Studied for Whole Body Retention and Thyroid Uptake of I^{131}

	Euthyroid	Hypo-thyroid	Hyper-thyroid	Complete Thyroid-ectomy	Partial Thyroid-ectomy	Suspected Hypopituitary Dwarf
Before TSH	12	1	0	2	1	1
After TSH	1	-	-	1	-	1

Total Patients = 17

Total Determinations = 20

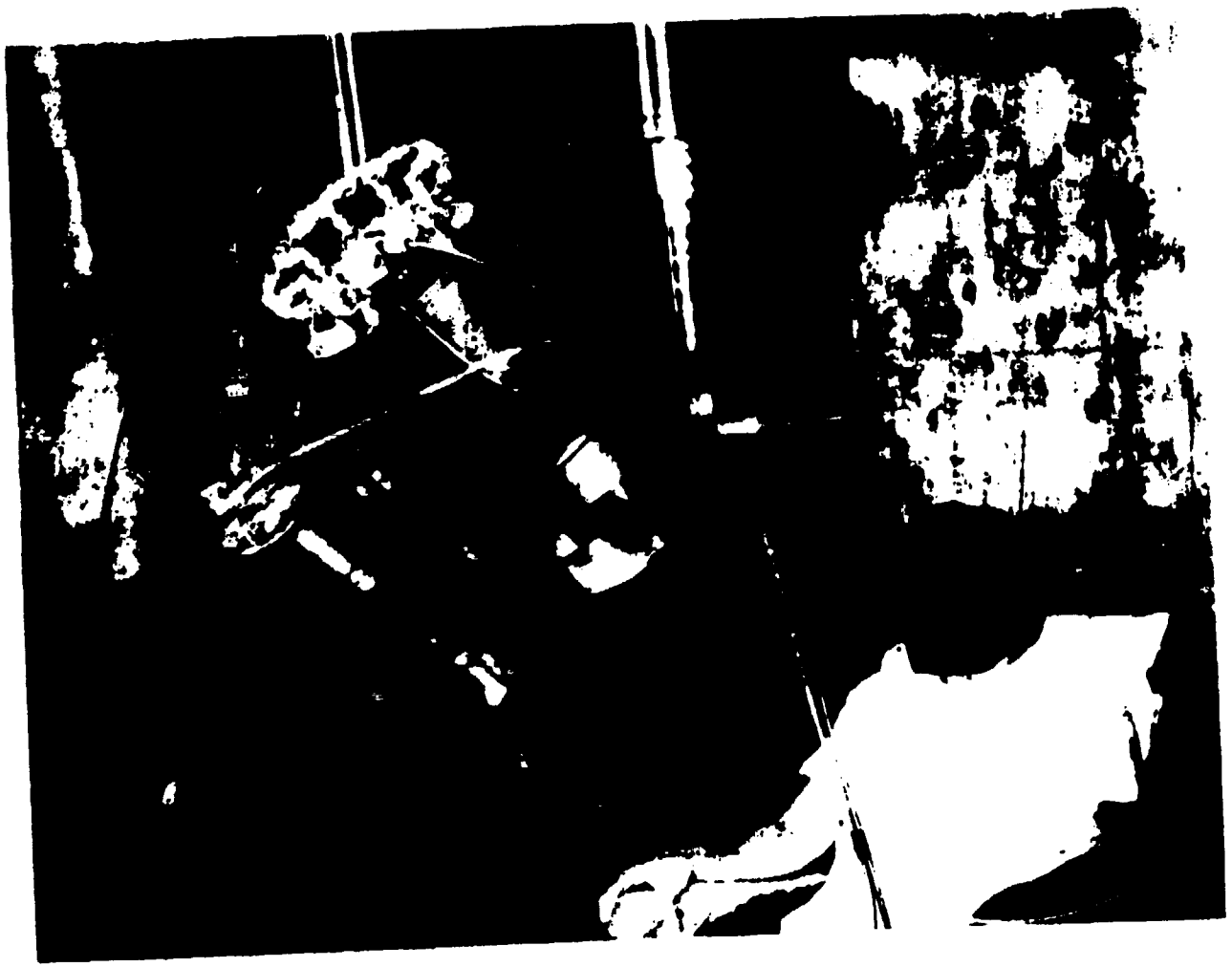

Fig. 1. Patient in position for whole body scintillometry with the 9 x 6 in. crystal. The 3 x 3 in. crystal is attached to the side of the housing of the larger one. The two crystals were positioned and operated individually.

The 3 x 3 in. crystal was at first used 10 cm away from the thyroid or phantom, but later was used at the more clinically acceptable distance of 25 cm. Neither the crystal nor the patient was filtered by the commonly used "A" or "B" filter (1). The data were processed using the following formulas:

LIQUID SCINTILLOMETER

$$\text{Per cent Retention} = \frac{\text{Patient counts - Patient background - Machine background}}{\text{Standard counts - Machine background}}$$

9 x 6 IN. SODIUM IODIDE CRYSTAL

$$\text{Per cent Retention} = \frac{\text{Gross patient counts - Machine background}}{\text{Initial patient counts (net), corrected for physical decay of } I^{131}}$$

3 x 3 IN. SODIUM IODIDE CRYSTAL

$$\text{Per cent Uptake} = \frac{\text{Neck counts - Machine background}}{\text{Standard counts - Machine background}}$$

Results and Discussion

Only 14 of the 20 determinations were completed with all 3 counters. These results are tabulated in Table 2, along with some pertinent clinical information. A typical retention versus uptake study is shown in Fig. 2 to demonstrate

TABLE 2. Whole Body Retention and Thyroid Uptake of I^{131}

Case No.	Sex	Age	Per cent Retention, Humco I, 24 hr	Per cent Retention, Humco I, 48 hr	Per cent Retention, 9 x 6 in. Crystal, 24 hr	Per cent Retention, 9 x 6 in. Crystal, 48 hr	Per cent Retention, 9 x 6 in. Crystal, Peak[a]	Per cent Uptake, 3 x 3 in. Crystal, 24 hr	Per cent Uptake, 3 x 3 in. Crystal, 48 hr	Per cent Uptake, 3 x 3 in. Crystal, Peak[a]	Comment
[redacted]	F	[redacted]	49	41	57	55	57	66	63	66	TSH
	F	[redacted]	47	35	53	42	42	34	40	40	Normal; chr. pyelitis
	F	[redacted]	35	34	43	40	40	31	40	40	Ca thyroid; compl. thyroidectomy?
	F	[redacted]	--	--	37	33	33	35	39	39	Normal; obesity
	F	[redacted]	48	33	48	31	--	--	--	--	Normal; obesity
	F	[redacted]	37	32	48	45	48	31	30	31	Normal, chronic pyelitis
	F	[redacted]	41	25	45	33	33	20	23	25[b]	Normal; obesity
	F	[redacted]	36	22	45	30	45	30	26	30	Normal
	M	[redacted]	35	22	34	24	34	30	35	30	[redacted]
	M	[redacted]	29	20	15	11	11	25	35	35	[redacted]
	F	[redacted]	20	20	27	25	--	--	--	--	Normal
	F	[redacted]	32	19	35	35	22	22	21	22	Normal
	M	[redacted]	31	19	33	24	33	27	25	27	Normal
	F	[redacted]	29	19	34	23	23	16	18	18	Normal
	F	[redacted]	27	19	37	22	--	--	--	--	Normal[d]
	M	[redacted]	25	17	27	20	20	15	17	17	Normal; obesity
	M	[redacted]	20	11	28	25	--	--	--	--	Normal; iodine [redacted] ade

TABLE 2 (continued)

[redacted]	M	[redacted]	24	8	18	10	--	--	--	--	Ca thyroid; compl. thyroidectomy?
[redacted]	F	[redacted]	37	7	25	4	--	--	--	--	Ca thyroid; compl. thyroidectomy?
[redacted]	M	[redacted]	14	6	20	12	--	4	4	4	TSH[e]

[a]Highest per cent pickup in neck.

[b]72 hours.

[c]Same as Case No. [redacted] before TSH.

[d]Same as Case No. [redacted] before TSH.

[e]Same as Case No. [redacted] after TSH.

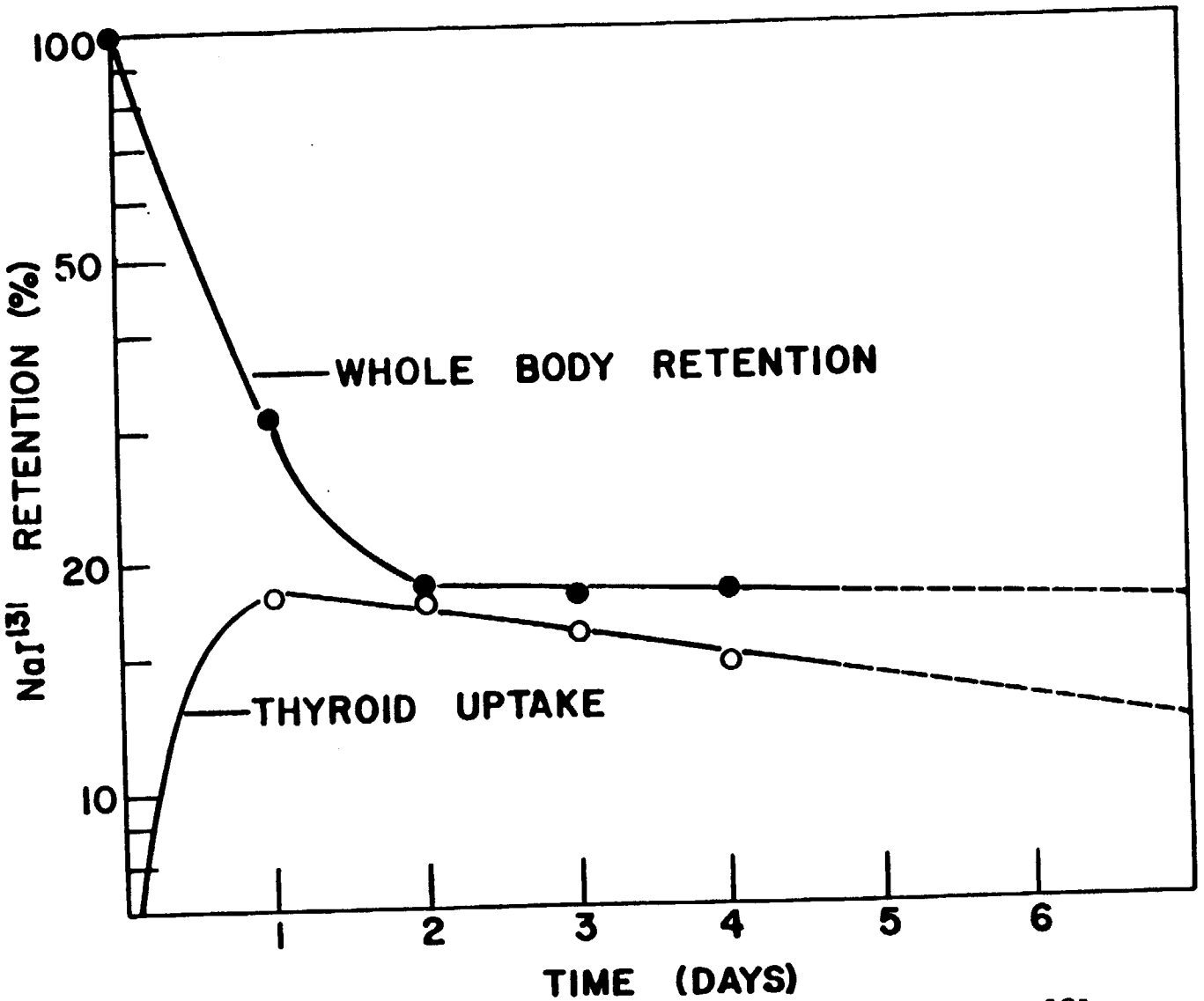

Fig. 2. A typical comparison between whole body retention of I^{131} and direct uptake by the thyroid.

the correspondence usually obtained between whole body retention and direct uptake by the thyroid. The whole body retention line is sometimes slightly lower than the thyroid uptake line. In this case, both lines appear to extrapolate to the same zero intercept of 21 per cent. (This case was not included in the original 20 studies.)

Figure 3 shows a graphic comparison of per cent whole body retention and per cent uptake by the thyroid, all at 48 hours after administration of the I^{131}. The data indicate reasonably good agreement among the three methods. The major disagreements were in Cases [redacted]. These cases were all obvious geometric problems. Case [redacted] was an extremely small thin woman who clinically was a low euthyroid who did not become clinically hyperthyroid with TSH, as indicated by direct counting over the gland. Case [redacted] showed renal retention and abdominal extrathyroid localization of the I^{131}. Cases [redacted] and [redacted] were the same patient, a [redacted] with a 30-year-old bone age, who seemed clinically not to respond to TSH. Case [redacted] was a bull-necked man, thyroidectomized completely 2 weeks before the test, whose neck count was 25 times his thigh count immediately after an intravenous injection of radioiodine and before any localization could have occurred. This disparity probably resulted from the disproportionate size of the vascular bed in the healing

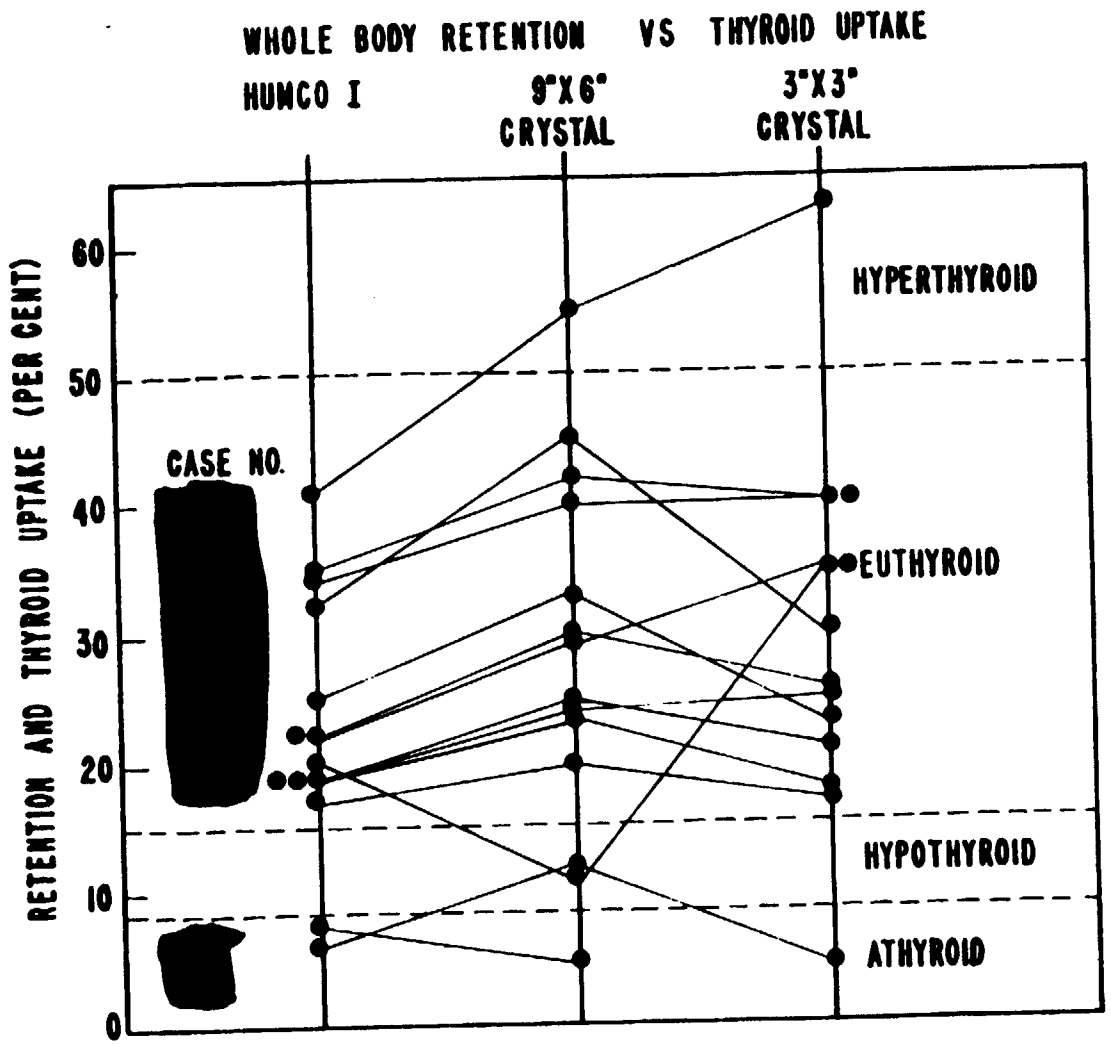

Fig. 3. A comparison of whole body retention and direct thyroid uptake of ingested I^{131} in 14 cases 48 hours after administration, when measured simultaneously with the scintillation counter, the whole body crystal spectrometer, and the collimated crystal placed over the thyroid gland.

thyroidal neck area. The correlation with biased clinical impressions (5) was best with the 4π liquid scintillometer. The liquid scintillator (Humco I) seemed to measure whole body retention better than the 9 x 6 in. crystal and thyroidal binding better than the 3 x 3 in. crystal, probably because of minimization of geometric variations. Correlation between the results of the two whole body counting methods at 48 hours was good.

From the practical point of view, a few crude time studies were made during the actual counting procedures. It was found that 14 minutes of the patient's time and 5 minutes the technician's time were needed per determination with the liquid scintillometer, while 15 to 20 minutes of the patient's time and about 45 minutes of the technician's time were needed to obtain a final result with the sodium iodide crystals.

These studies suggest that radioassay of NaI^{131} retention by the whole body liquid scintillometer is a valid, simple, and facile means of determining thyroidal iodine binding and hence thyroidal function. Additional studies (this report) in rats and humans, under various experimental and clinical conditions, extend these observations and support this conclusion by showing that the per cent retention of orally administered iodine in the whole body depends upon

relative need for iodine, thyroidal and pituitary activity, and renal function.

References

(1) M. Brucer, Oak Ridge Institute of Nuclear Studies Report ORINS-19. Available from Technical Information Services, Washington, D. C. (1959).

(2) E. C. Anderson, In: Liquid Scintillation Counting, F. N. Hayes and C. G. Bell, Jr., eds., Pergamon Press: New York, pp. 211-219 (1958).

(3) W. H. Langham, In: Liquid Scintillation Counting, F. N. Hayes and C. G. Bell, Jr., eds., Pergamon Press: New York, pp. 135-149 (1958).

(4) E. C. Anderson and M. A. Van Dilla, I. R. E. Trans. Nucl. Sci. NS-5, 194 (1958).

(5) S. C. Werner, ed., The Thyroid: A Fundamental and Clinical Test, Harper and Brothers: New York (1955).

Clinical Applications of Whole Body Scintillometry. III. Whole Body Retention of Iodine^{131} as a Method of Studying Thyroid Function in Man

(C. C. Lushbaugh and D. B. Hale)

Introduction

An apparently good correlation between total body retention of I^{131} (measured by whole body counting) 3 days after oral administration and thyroid uptake (measured in the conventional way) in the same patient seems to justify further study of total body retention as a method of determining thyroid function. The previous report (1) suggests that the second of the two components of the retention curve expresses thyroid binding of I^{131} and, therefore, is indicative of the level of thyroid activity. Furthermore, the observation that food and iodine deprivation in rats increased total body retention of I^{131} and decreased its rate of excretion (2) provides more evidence that iodine metabolism is manifested by changes in whole body retention of an I^{131} tracer. Additional observations on humans are reported here to demonstrate further how the I^{131} retention function changes with disease, chemotherapy, and metabolic status.

Methods and Materials

The method of whole body counting and other techniques used in these studies is described fully in the preceding paper (1). Data on some of the patients described in that study are used in the present paper along with additional data from other patients with normal and abnormal thyroid function. Only the whole body liquid scintillation counter was used to measure body retention in the additional patients. The number of athyroid children studied was increased to 6, and 1 proven hyperthyroid and 1 hypothyroid case have been studied. A total of 35 patients have been studied after oral NaI^{131} administration; 20 have been studied after intravenous I^{131}-thyroxine and 6 after intravenous I^{131}-triiodothyronine. Two additional patients were given Lugol's solution to study its effect on the rate of excretion of bound I^{131}. Iodine^{131} retention by 22 of the apparently normal patients was followed as long as possible to try to establish the mean slope of the final exponential decay rate.

Results and Discussion

The results of these studies are presented graphically in Figs. 1-6. The total body I^{131} retention patterns, depicted in Fig. 1, show that the retention curve can vary widely from the normal in diseased states and may be influenced

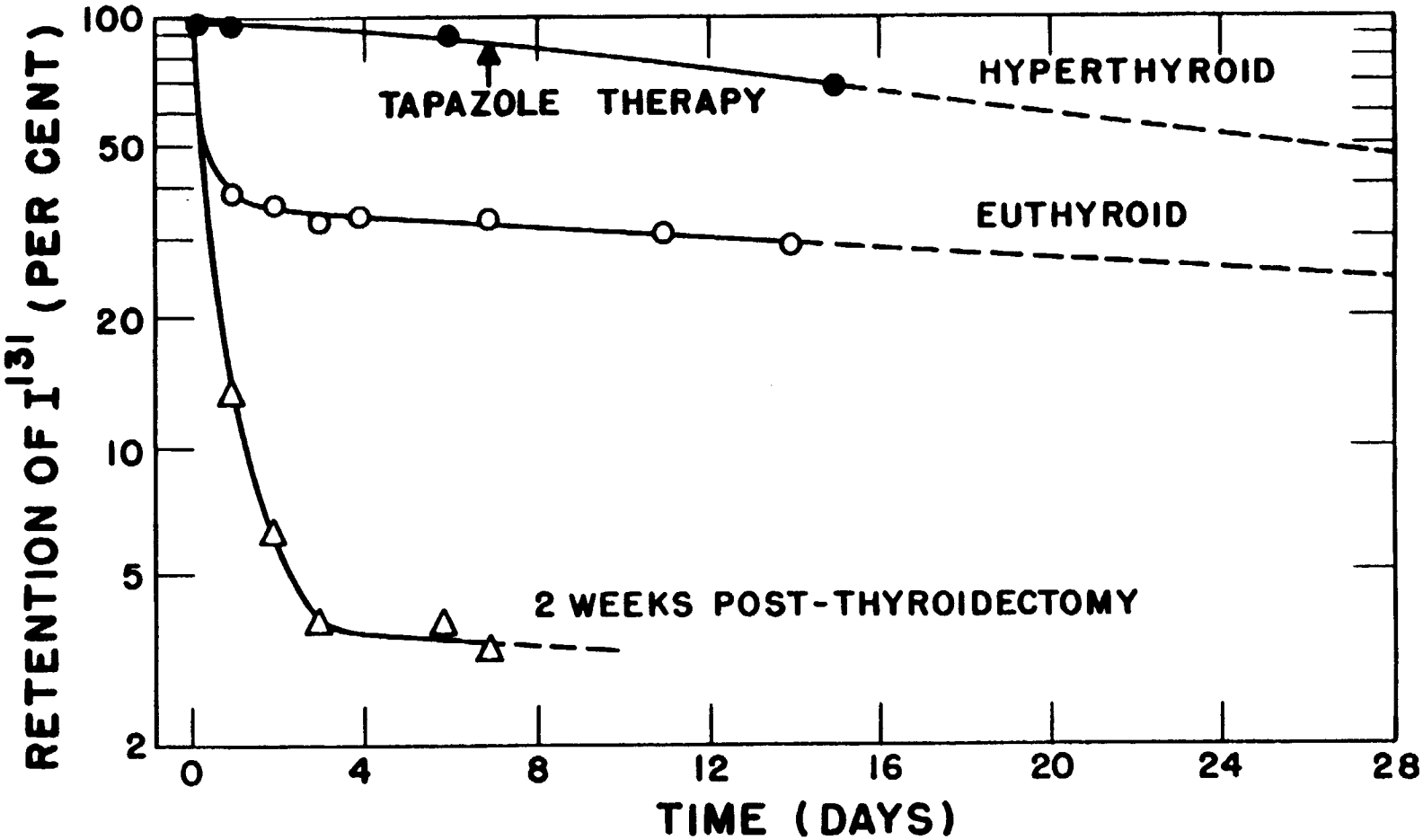

Fig. 1. Whole body retention of I^{131} in various thyroid conditions following oral administration as NaI.

by therapy (e.g., tapazole). In both normal and abnormal cases, the whole body retention function is the sum of 2 exponentials, as indicated in Fig. 2, which shows I^{131} retention in euthyroid and athyroid children.

The first exponential (A) represents the rate of urinary excretion of unbound iodide, as shown by quantitative urine collections during the first 24 hours after oral administration of the tracer. All but a minute trace of the iodine excreted during this time is inorganic (3) and represents effective renal competition with the thyroid for unbound I^{131} in the serum. These data also show that the half-times of excretion of unbound iodine by normal children and children thyroidectomized for cancer were approximately the same, 8.4 and 9.6 hours, respectively. In all the cases studied, this half-time varied between 7 and 22 hours. It appears to be prolonged by poor renal function as in chronic pyelonephritis and other renal diseases. The second exponential (B) represents the amount of orally administered I^{131} incorporated in the bound iodine cycle (3,4). The slope of the second exponential reflects the rate of urinary loss of iodide freed from the bound iodine pool as the labeled hormones are utilized and degraded.

An attempt was made to use retention data from

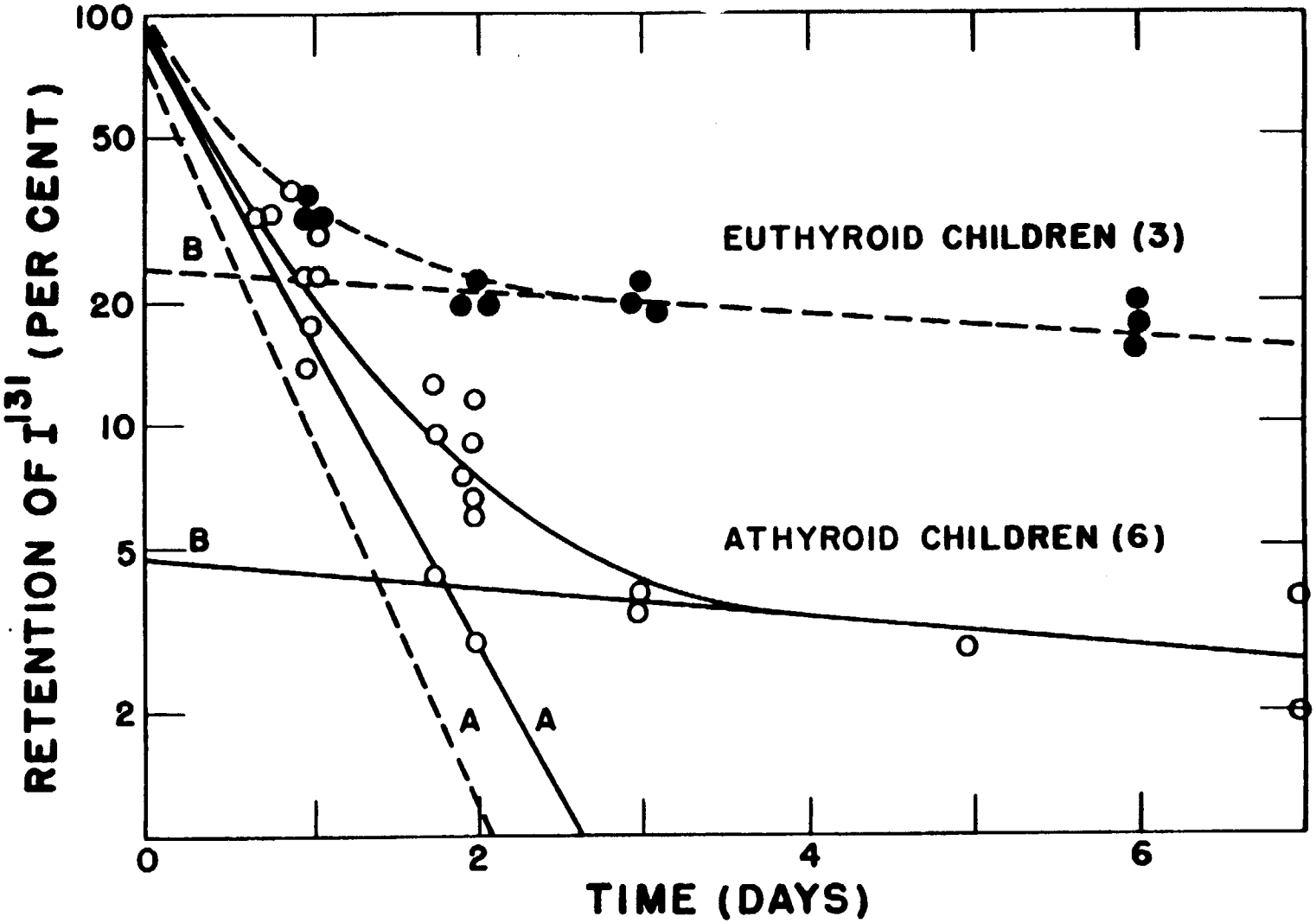

Fig. 2. Whole body retention of orally administered NaI^{131} in normal and completely thyroidectomized children.

22 supposedly normal patients to establish the average normal rate of turnover of the bound iodine pool (component B). Collection of data, however, was too sporadic to permit establishment of the half-time with satisfactory accuracy. It was possible only to say that the slope appeared to represent a half-time of between 40 and 50 days, corresponding to a rate of bound iodine loss of 1.4 to 1.7 per cent per day. A half-time of about 50 days is consistent with values reported in the literature (3,4) and is assumed to be the proper one to use in this report.

Figure 3 shows the average I^{131} whole body retention by 6 normal subjects when the tracer was administered intravenously as I^{131}-triiodothyronine, and Fig. 4 shows its average retention when injected into 20 normal people as I^{131}-thyroxine.

When the tracer I^{131} is introduced into the cycle as a label on the thyroid hormones, the whole body retention patterns change and their exponential components appear to be the utilization rates of these products of thyroidal activity. Two components are seen following administration of labeled triiodothyronine (Fig. 3), a rapid early one (A) representing the amount of I^{131} tracer excreted after intracellular degradation of the hormone, and another (B) showing the amount of tracer recycled through the thyroid bound

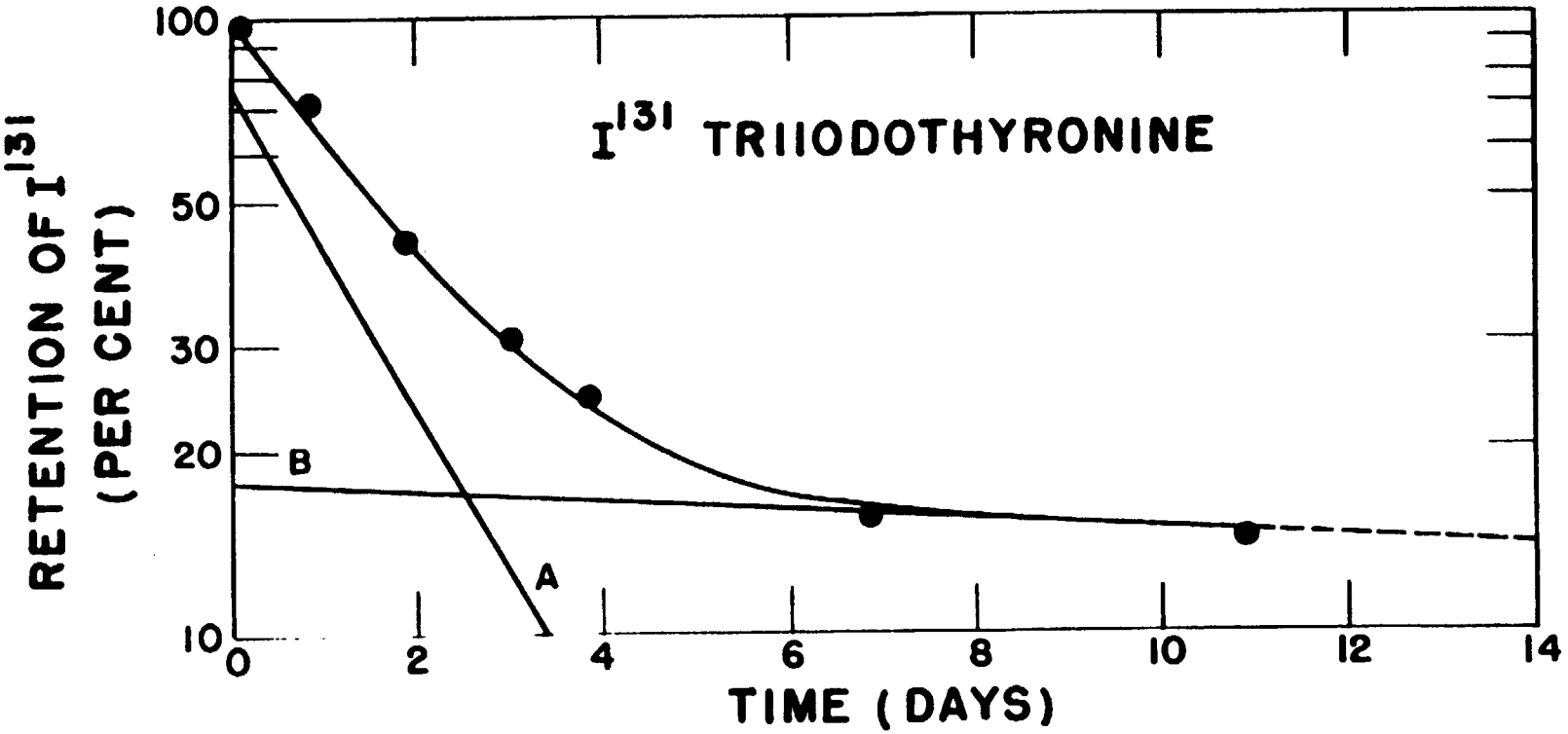

Fig. 3. Whole body retention of I^{131} after intravenous administration as I^{131}-triiodothyronine to euthyroid patients.

iodine pool. The first exponential has a half-time of 1.4 days and the second a half-time of about 50 days, as previously reported for the rate of turnover of bound iodine. The rate of iodine excretion in (A) appears to be determined by the rate of utilization of triiodothyronine which frees iodide (3,4).

The total body retention line following administration of I^{131}-thyroxine (Fig. 4) appears to show three exponentials; the first (A) with the same slope as the exponential (A) in the triiodothyronine study; a second (B) with a 7.5-day half-life; and a third (C) with approximately a 50-day half-life. Exponential (B) may represent the rate of urinary excretion of iodide after intravenous breakdown of thyroxine to triiodothyronine with the liberation of an iodide ion (3). This rate of excretion is determined by the rate of degradation of thyroxine which the line, therefore, represents. Presence of the first exponential (A) implies contamination of the labeled thyroxine with labeled triiodothyronine, since such a rapid utilization rate (1.4 day half-time, see Fig. 4) could not be determined by whole body counting if the compound being used was formed at a slower production rate (7.5-day half-time) by a "mother" substance. Exponential (C), again, represents the fraction of iodide freed after thyroxine and triiodothyronine utilization, and recycled through the thyroid

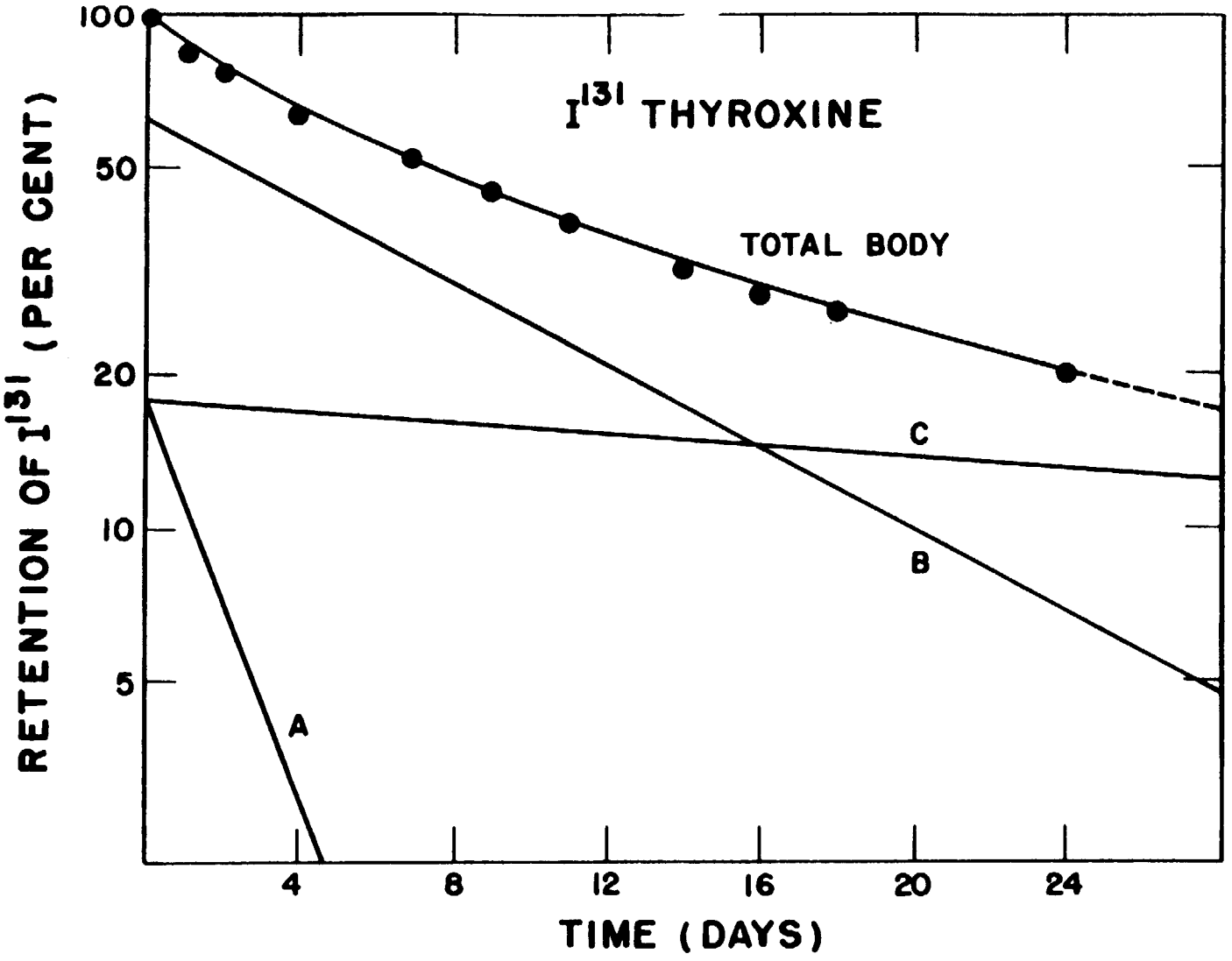

Fig. 4. Whole body retention of I^{131} after intravenous administration of I^{131}-thyroxine to euthyroid patients.

pool instead of being excreted at once by the kidneys.

It would seem from these studies that the final whole body retention line, therefore, represents the binding capacity of the thyroid and cycling of the I^{131} tracer as iodinated thyroglobulin, thyroxine, triiodothyronine, diiodotyrosine, and free iodide as diagrammed by Riggs (3) and Werner (4).

Figure 5 shows how the rate of turnover of the bound iodine pool may be increased in a normal person by increasing intake of stable iodine. Dilution of the I^{131} tracer in the enlarged free iodide pool decreases the per cent of tracer that can be bound by the thyroid and forces its excretion along with the increased urinary excretion of the stable iodide. In this case, 150 mg of iodine (as Lugol's solution) per day in an 85-kg woman decreased the half-time of this exponential from about 55 to about 20 days, so that the apparent turnover rate changed from 1.3 to 3.5 per cent per day.

Figure 6 shows NaI^{131} uptake and retention by the same (hypothyroid) patient before and after administration of thyroid stimulating hormone (TSH). Treatment was administered on the day preceding ingestion of the I^{131}. The data show that TSH definitely increased the relative size of the bound iodine pool and suggest that whole body retention of I^{131}

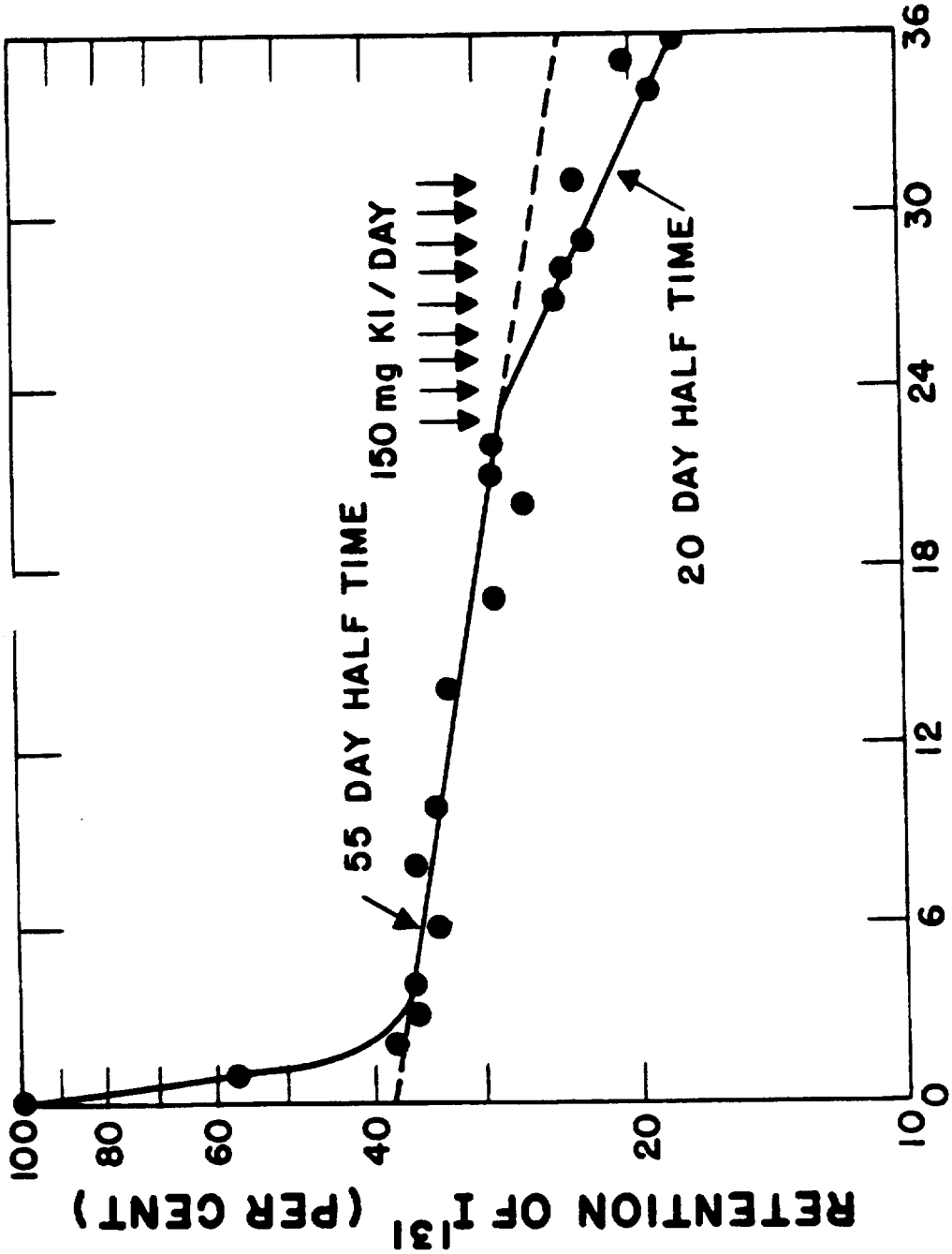

Fig. 5. Effect of a large intake of stable iodine on the retention of I^{131} in the bound iodine cycle.

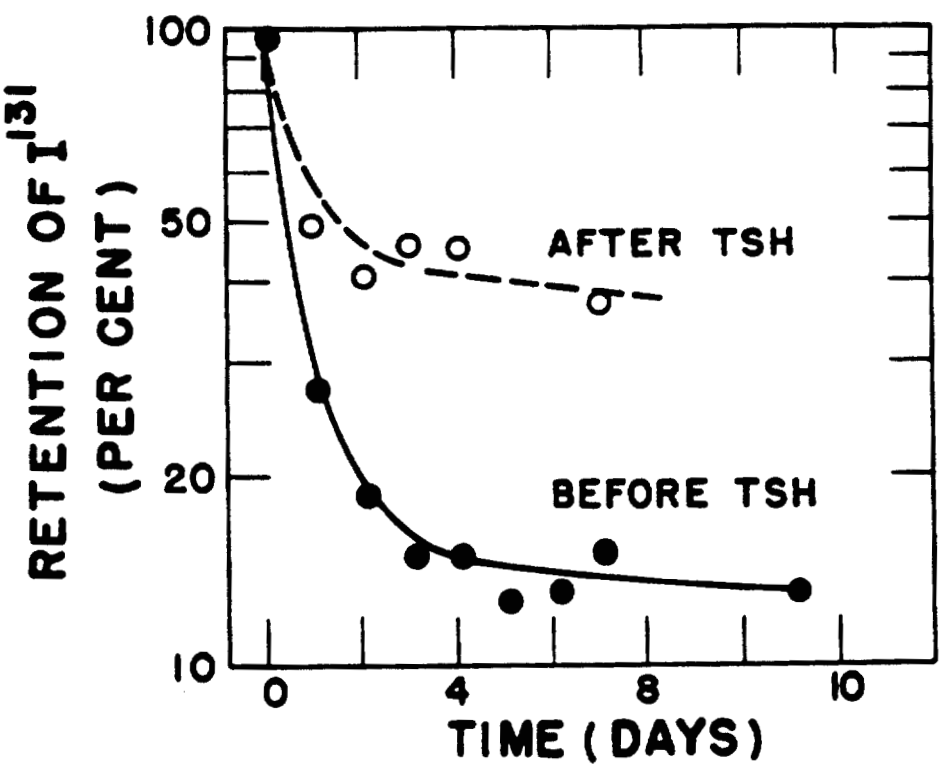

Fig. 6. Effect of thyroid stimulating hormone on the whole body retention of I^{131}.

is an indication of the thyroid binding activity at the time of absorption of the tracer.

When considered altogether, these studies appear to support the following conclusions:

1. Unbound I^{131}, after gastric absorption, is excreted rapidly by the kidneys (half-time 7 to 10 hours).

2. Bound I^{131}, after entrapment by the thyroid gland, is lost slowly by the urinary route (half-time about 50 days).

3. The rate of urinary loss of I^{131} from the bound iodide pool is determined by (a) daily iodide intake; (b) size of free iodide pool (specific I^{131} activity); (c) thyroid binding activity; (d) rate of conversion of thyroglobulin to thyroxine; and (e) competitive ratio of renal function to thyroid function.

4. The per cent of oral tracer dose retained by thyroid binding is dependent upon the same factors as in (3) and expresses the equilibrium state of the patient's iodine metabolism, which can be studied by whole body I^{131} retention measurements.

References

(1) C. C. Lushbaugh and P. New, Clinical Applications of Whole-body Scintillometry. II. Comparison of Whole-body retention of NaI^{131} with Thyroid Uptake as Measured by a NaI Crystal. This report.

(2) C. C. Lushbaugh and D. B. Hale, Iodine^{131} Retention in Normal and Starved Rats. This report.

(3) D. S. Riggs, Pharmacol. Revs. 4, 284 (1952).

(4) S. C. Werner (ed.), The Thyroid. A Fundamental and Clinical Text. Harper and Brothers, New York (1955).

Iodine^{131} Retention in Normal and Starved Rats (C. C. Lushbaugh and D. B. Hale)

Introduction

Iodine^{131} uptake by the thyroid gland of experimental animals is difficult to determine because of the small size of the gland in relation to the counting apparatus and the resulting large errors due to geometrical effects from movement of the animal during measurement (1,2). Thyroid uptake in small animals is, therefore, commonly done by removing the thyroid and radioassaying solutions of the gland obtained after digestion (3,4) or by counting the whole organ in a Texas well counter (5). A scintillation well counter large enough to hold an entire rat makes possible repeated reproducible measurements of iodine retention in the whole body without the need for sacrificing the animals. The Los Alamos small animal counter, described in detail elsewhere (6), has proved to be ideal for this purpose. If the ratio of thyroid and extrathyroid iodide in the rat becomes constant as it does in the human being (7), then thyroid uptake in rodents could be determined by whole body counting, as in the human (8,9). The following experiments were done to investigate this possibility.

Methods and Results

Eighteen Sprague-Dawley male rats, weighing approximately 300 g, were given 1 μc NaI^{131}. Nine received the isotope orally and were allowed food ad libitum. The other nine were given the dose intraperitoneally and were starved throughout the experiments. A reference standard was made using the dose of 1 μc NaI^{131}. The rats were counted at once after administration of the dose and at approximately 4, 10, 24, 50, 60, 70, 84, 96, 105, 120, 144, 168, and 195 hours afterward. The data were expressed as per cent of the standard dose retained (converted to per cent of the original dose for each rat).

On the 6th day after administration of the dose, the starving rats were killed and their thyroid glands removed by extirpation of the trachea, thyroid, and adjacent tissue. The animals fed normally were not killed until the 8th day. After removing the thyroid glands, the carcasses were recounted in order to determine the partitioning of the retained per cent of original dose in the thyroid and extrathyroid tissues.

Figure 1 shows the average daily per cent retention of NaI^{131} for the two groups of rats plotted semilogarithmically. After 3 days, the per cent retention began to decrease as a single exponential line. When this exponential line (designated B)

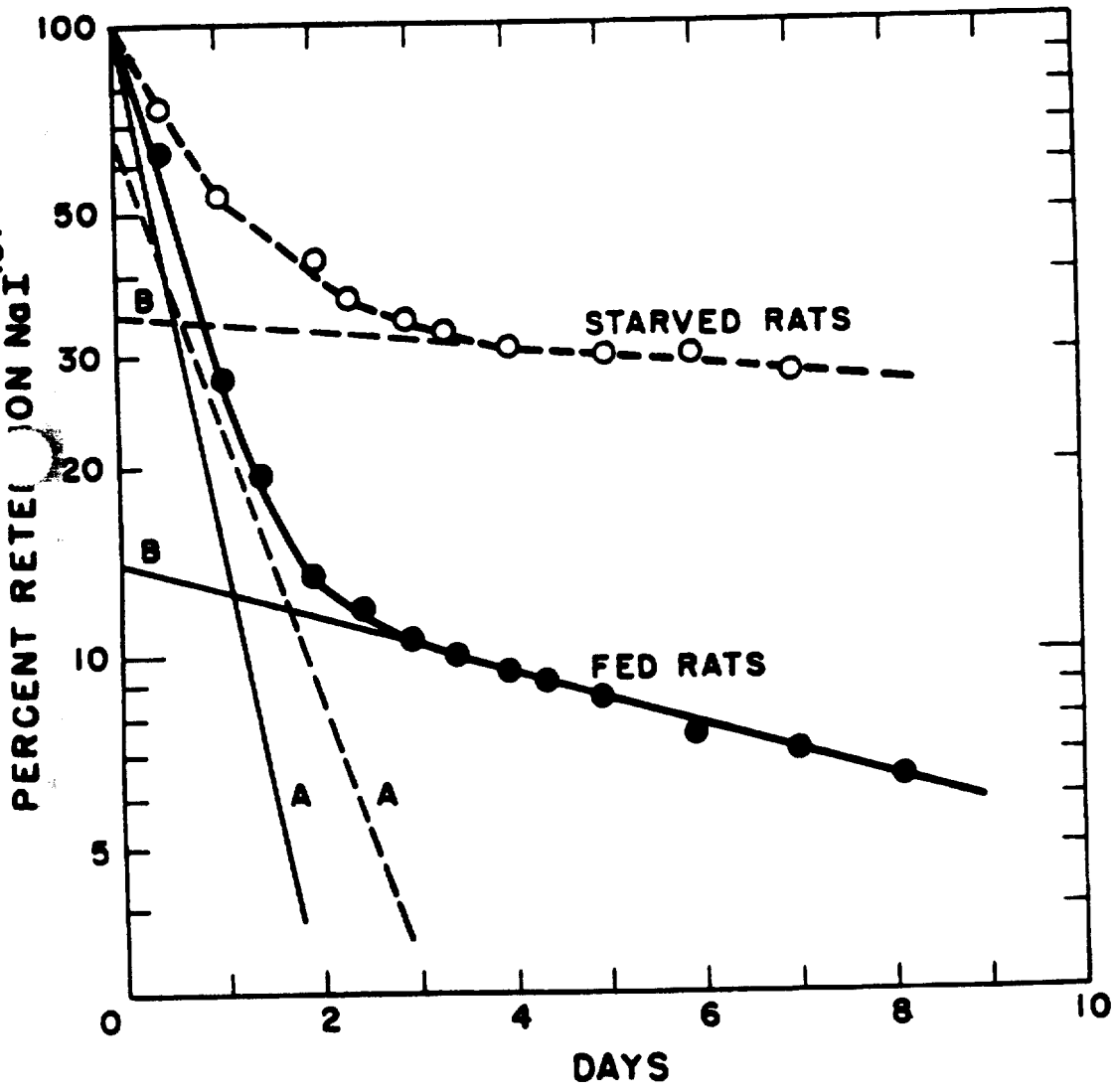

Fig. 1. Retention of NaI^{131} in normal and starved rats, determined by in vivo whole body counting.

was subtracted from the (heavier) total retention curve, a second exponential line (A) was found. Serum radioactivity was high in the nonprotein fraction of the blood during time exponent A was dominant, but present only in the protein-bound fraction when exponent B was operational. The parameters of the excretion functions for the individual animals are summarized in Table 1. Assuming the exponentials of the retention functions represent metabolic pools, the turnover rate and mean residence time for I^{131} in each compartment are given. In determining the averages, rats 2 and 8 in the normal group were omitted on the basis that the size of compartment B, as judged by the zero intercept, placed these rats (which appeared ill late in the experiment) with the "starved" group. They were also omitted from the averages in Table 2, which shows the I^{131} content (in per cent of administered dose) of normal and starved rats before and after post mortem thyroidectomy. The difference in body content before and after thyroidectomy is assumed to be a measure of the extrathyroidal iodine. In terms of per cent of administered dose, whole body retention of I^{131}, extrathyroidal iodine, and thyroid bound iodine were all increased by starvation.

Discussion

The results show that the normal rat "picks up" 8 to

TABLE 1. Parameters of Whole Body NaI^{131} Retention in Rats

Group	Zero Intercepts (%)		Half-Times (hrs)		Turnover (%/hr)		Mean Time (hrs)	
	A*	B*	A	B	A	B	A	B
Normal Rats								
1**	90	9.9	9	185	77	0.37	13.0	267
(2)	73	29.0	9	189	77	0.37	13.0	273
3	88	12.0	9	149	77	0.47	13.0	215
4	92	8.3	8.5	159	82	0.44	12.3	229
5	85	15.0	9	174	77	0.40	13.0	251
6	89	11.0	7.5	157	92	0.44	13.3	227
7	88	12.5	9.5	207	73	0.33	13.7	299
(8)	72	28.0	9.5	160	73	0.43	13.7	231
9	77	23.0	10	164	69	0.42	14.4	237
Average	87	13.0	9	171	78	0.40	13.0	248
Starved Rats								
1	62	38	12	585	58	0.12	17.3	844
2	69	31	12	865	58	0.08	17.3	1248
3	82	26	11	515	63	0.13	15.9	743
4	67	33	8	225	87	0.31	11.5	325
6	73	27	13	271	53	0.26	18.8	391
7	64	36	12.5	307	55	0.23	18.0	443
9	57	34	11.5	222	60	0.31	16.6	320
Average	65	35	11.4	427	62	0.21	16.5	616

* A = First exponential line (rate of excretion of unbound iodine);
B = Second exponential line (rate of excretion of bound iodine).

** ( ) Omitted from average.

TABLE 2. Effect of Starvation on Whole Body Retention and Distribution of I^{131} in Rats

Group	Whole Body Retention: Before Thyroidectomy (%)	Whole Body Retention: After Thyroidectomy (%)	Thyroid Concentration: % of Original Dose	Thyroid Concentration: % of Retained Dose
Normal Rats (195 hours)				
1	5.1	4.1	1.0	20
2	(14.8)*	(9.3)	(5.5)	(37)
3	5.4	3.6	1.8	33
4	3.8	2.4	1.4	37
5	7.3	5.0	2.3	32
6	4.7	3.0	1.7	36
7	6.6	3.7	2.9	44
8	(12.8)	(6.5)	(6.3)	(49)
9	10.4	5.9	4.5	43
Average	6.2	4.0	2.2	35
Starved Rats (143 hours)				
1	32.0	18.6	13.6	42
2	25.0	15.0	10.0	40
3	19.7	14.0	5.7	29
4	20.0	12.4	7.6	38
6	18.6	7.5	11.1	60
7	25.5	12.4	13.1	51
9	25.0	13.4	11.6	46
Average	23.7	13.3	10.4	44

*( ) Omitted from averages.

23 per cent (average 13 per cent) of an ingested I^{131} tracer dose. Starvation apparently alters the amount which is concentrated by the thyroid and its rate of turnover. Starva-

those investigators felt justified on this basis to diagnose hyper- and hypothyroidism, respectively, it would seem in retrospect that these changes in uptake may reflect only the different specific activities of the unbound iodide pools after radioactive labeling and not different thyroid activities. Before such diagnoses can be made by any radioisotopic method, all groups of animals should be maintained on the same known oral intake of stable iodine. The observation that starving rats conserve orally administered I^{131} and hold a greater percentage in the gland may help to explain the phenomenon of "increased thyroid binding" of radioiodine in rats exposed to large doses of whole body ionizing radiation (4,12). The increases found by those investigators would seem to parallel the degree of anorexia and self-starvation found commonly in the irradiated rodent (13). The increased retention of I^{131} during starvation is remarkably similar to the increased retention of sodium (14) and potassium (15) in starving heavily irradiated rats.

Studies of whole body I^{131} retention in rodents are feasible using in vivo counting techniques. If iodine intake is maintained at a constant level, the per cent of retention can be used to evaluate thyroid binding activity in small animals.

References

(1) D. L. Jofte, Int. J. Appl. Radiation and Isotopes 4, 227 (1959).

(2) A. Albert, Endocrinol. 48, 334 (1951).

(3) M. E. Morton, I. Perlman, E. Anderson, and I. L. Chaikoff, Endocrinol. 30, 495 (1942).

(4) D. D. Ulmer, L. B. Perkins, and J. G. Kereiakes, Rad. Res. 11, 810 (1959).

(5) H. W. Johnson and A. Albert, Endocrinol. 48, 669 (1951).

(6) K. T. Woodward, C. R. Richmond, and W. Langham, Proceedings of the Health Physics Society, p. 79 (1956).

(7) S. C. Werner, ed., The Thyroid: A Fundamental and Clinical Text, p. 45. Harper and Brothers: New York (1955).

(8) C. C. Lushbaugh and P. New, this report. Clinical applications of whole body scintillometry. II.

(9) C. C. Lushbaugh and D. B. Hale, this report. Clinical applications of whole body scintillometry. III.

(10) B. Catz, I. E. Rawi, and E. Geiger, Am. J. Physiol. 172, 291 (1953).

(11) C. Cohn, D. Joseph, L. Oliner, and J. V. Morton, Endocrinol. 62, 251 (1958).

(12) J. B. Storer and H. C. Simonson, Proc. Soc. Exptl. Biol. Med. 90, 369 (1955).

(13) D. E. Smith, E. B. Tyree, H. M. Patt, and E. Jackson, Proc. Soc. Exptl. Biol. Med. 78, 774 (1951).

(14) C. C. Lushbaugh, J. Sutton, and C. R. Richmond, Rad. Res., submitted for publication.

(15) C. C. Lushbaugh, D. B. Hale, and T. T. Trujillo, this report. Additional observations on electrolyte and water loss in radiation damage: Potassium-42 and HTO.

The Question of Sodium Loss in the Intestinal Death Syndrome of Radiation Damage (C. C. Lushbaugh, J. Sutton, and C. R. Richmond)

Introduction

Many hypotheses have been formulated to explain the phenomenon of "intestinal death" from radiation damage. Quastler (1) concluded that the two most probable causes of death were the "action of proteolytic enzymes and/or loss of water and electrolytes." While supported indirectly by innumerable clinical and laboratory observations of "cholera-like" diarrhea, severe dehydration, and systemic toxemia, and by amelioration of damage by intestinal shielding (2), intestinal extirpation (3), and massive saline infusion (4,5), little or no directly supportive data have been found for either of these causes of death. An experimental evaluation of the concept of death being due to electrolyte or water loss appeared possible by determination of sodium, potassium, and water turnover rates (6,7) in normal and irradiated animals. The experiments reported here attempt to test directly the hypothesis that large acute doses of whole-body irradiation affect distribution and excretion of body sodium.

Methods and Results

Effect of Radiation on Gross Distribution of Sodium.--

The first experiment was designed to show the effect of 100 rads of 250 KVP X irradiation on gross distribution of a tracer dose of Na^{22}. Since gastric retention and apparent starvation are characteristic of heavily irradiated animals, it was necessary to study also the effect of food deprivation. A group of 12 rats was divided into control and irradiated subgroups of 6 animals each. Three animals from each subgroup were deprived of food beginnning 12 hours prior to the experiment, and the others were allowed food ad libitum. Three animals from each subgroup were irradiated with 2100 rads of X rays (at a dose rate of 57 r/min) and all animals, including the fasted and nonfasted unirradiated controls, were given (via stomach tube) 0.47 μc Na^{22} in 1 ml of physiologic saline. Immediately after Na^{22} administration all animals were counted in the small animal liquid scintillation counter (8). Twenty-four hours later they were counted again to determine the per cent retention of the Na^{22} in terms of the original dose. They were sacrificed immediately after counting and the per cent of the administered dose retained in the gastrointestinal tract, stomach, small plus large intestines, blood (10 ml), and carcass determined by counting in the same counter. The data showing the comparative

effects of fasting and/or 2100 rads of irradiation on the gross distribution of Na^{22} are given in Table 1.

TABLE 1. COMPARATIVE EFFECTS OF FASTING AND/OR 2100 RADS X IRRADIATION ON 24-HOUR GROSS DISTRIBUTION OF Na^{22} IN RATS*

	Whole-Body Retention	Carcass	G. I. Tract	Stomach	Intestines	10 ml of Blood
Controls						
Fed	84	68	9	1	8	7
	85	70	9	1	8	7
	81	66	9	1	8	7
Fasted	93	76	7	1	6	7
	93	77	8	1	7	7
	91	75	8	1	7	7
2100 rads						
Fed	98	55	38	31	8	5
	97	55	36	30	7	5
	99	31	67	63	5	2
Fasted	90	73	8	1	7	7
	96	76	10	1	9	8
	96	77	9	1	8	8

*All results are in terms of per cent of administered dose.

Effect of Starvation on Sodium Retention.--Simultaneously with the above experiment, 12 animals were used to determine effect of starvation alone on rate of excretion of body sodium. All animals were given a tracer dose of Na^{22} via stomach tube and left on ad libitum feeding for 3 days. At that time, half

of the group was left on continuous feeding and the other half starved for an additional 6 days. Throughout the 9-day experiment the total Na^{22} retention of both groups was determined by daily whole-body counting. The averaged data showing effect of starvation alone on sodium retention or excretion are shown in Fig. 1.

Effect of Irradiation on Sodium Retention.--Since heavily irradiated animals show gastric retention or fail to eat, an experiment was designed to compare Na^{22} retention in irradiated animals with that of starved unirradiated controls to see if exposed animals showed indication of accelerated body sodium loss. Five groups of at least 6 animals each were given Na^{22} ($\sim$1 μc per rat) via stomach tube and kept on ad libitum feeding for 3 days. Their retained Na^{22} was measured daily via whole-body counting. During the third day, randomly selected groups were given 1000, 2000, 5000, and 8000 rads of 250 KVP X rays (at a dose rate of 57 r/min) and one group was kept as a control. Periodic measurement of Na^{22} retention was continued until death of the irradiated groups. The average retention of each group as a function of time is shown in Fig. 2. A single line fits the data for all groups.

Effect of Irradiation on Total Exchangeable Sodium.--Another experiment was designed to determine whether acute

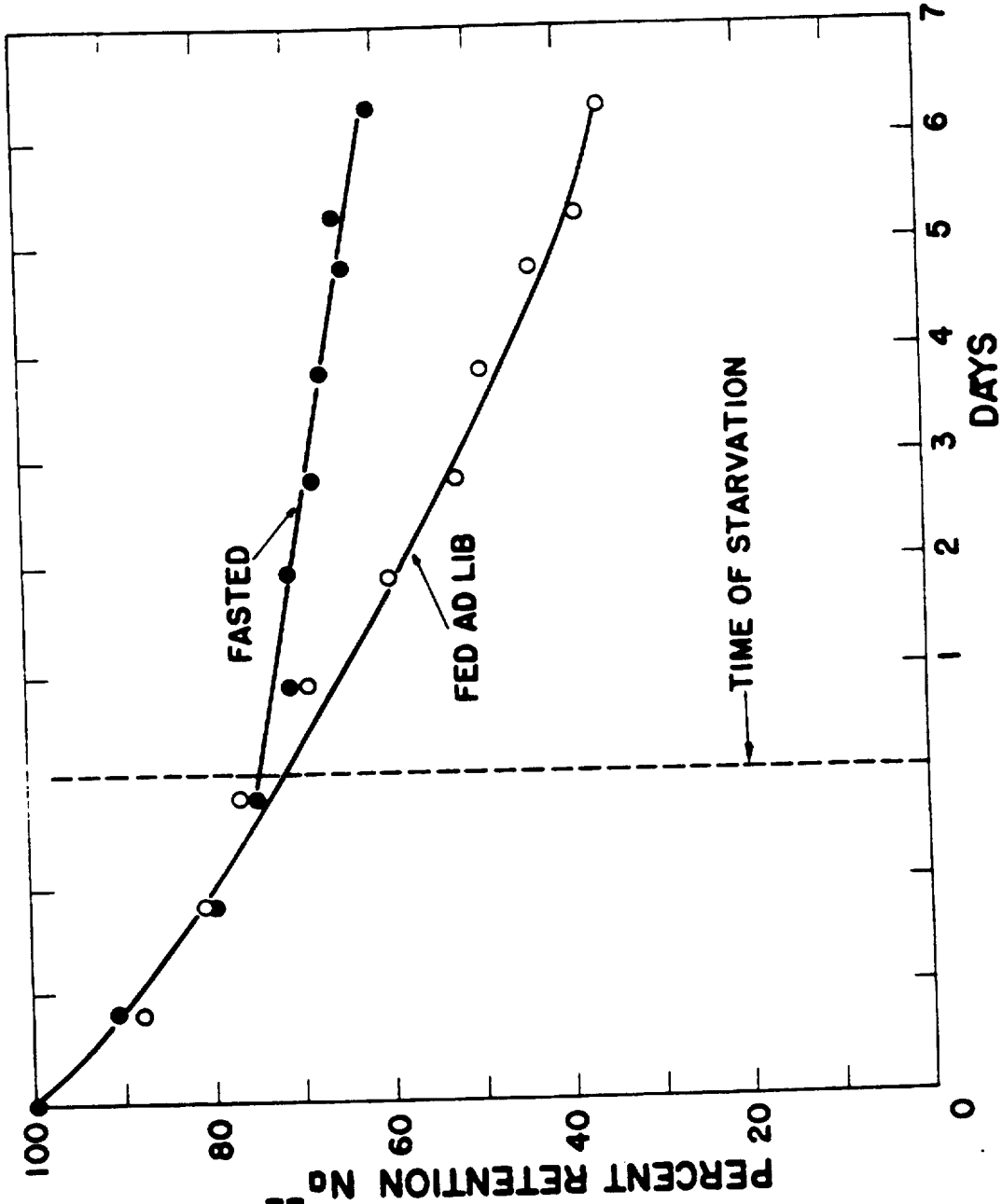

Fig. 1. Effect of starvation on retention of body sodium.

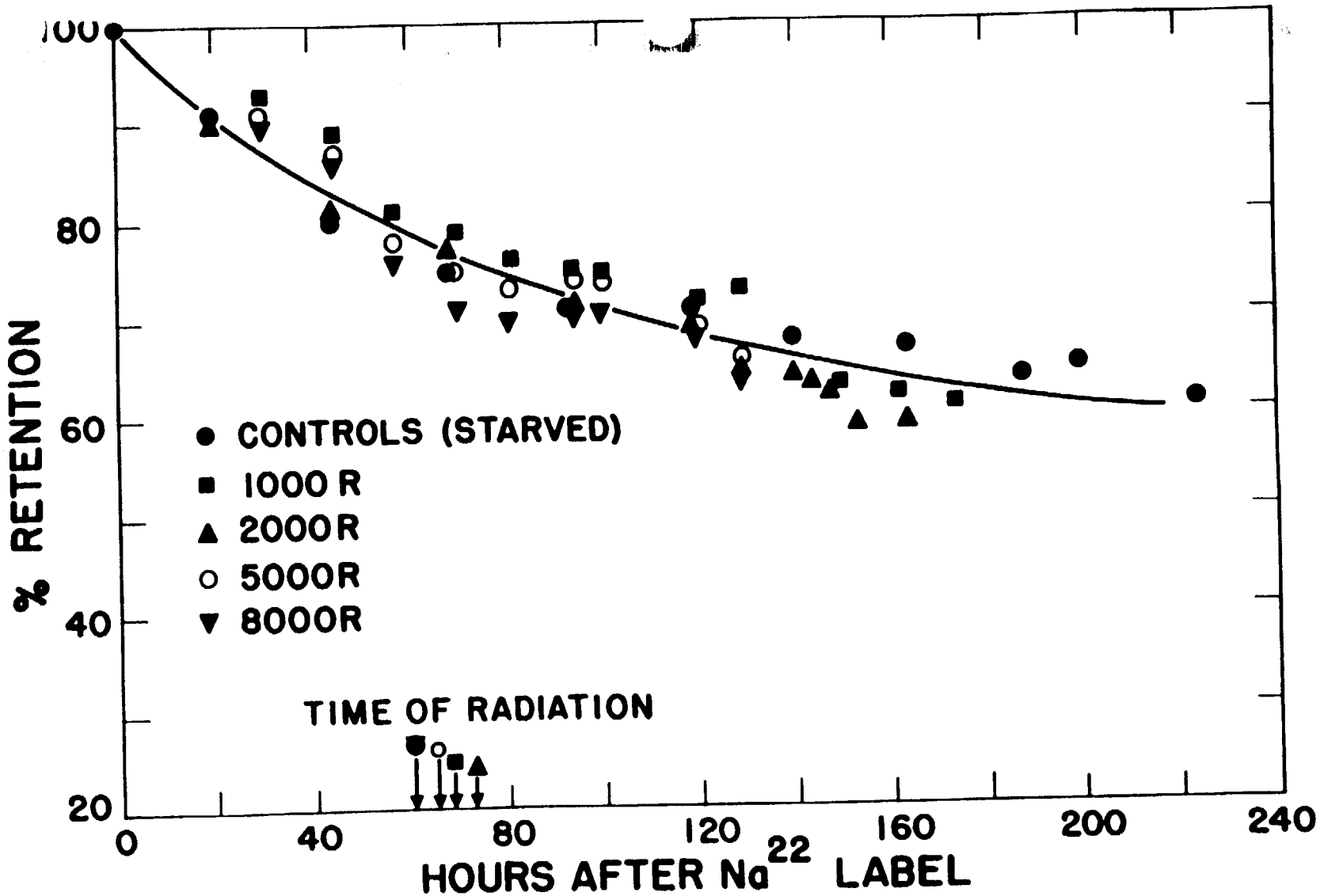

Fig. 2. Effect of 1000 to 8000 rads of 250 KVP X-ray exposure on rate of retention of Na^{22} by fasting rats.

radiation produced significant changes in total exchangeable sodium, even though the per cent retention of Na^{22} did not appear to be altered. Three groups of 8 rats each were given Na^{22} by mouth and kept on ad libitum feeding for approximately 2 days, after which they were deprived of food. At the same time one group was given an acute exposure of 700 rads and another 2100 rads of 250 KVP X rays. The third group was kept as a control. Throughout the experimental period, total retention of Na^{22} was measured daily and urine samples collected. The specific activity of urinary sodium was derived from the Na^{22} counts and from the total sodium determined by flame photometry. From these data, the total exchangeable sodium was estimated by the following expression:

$$Na_e = \frac{\text{Whole Body Activity (d/sec)}}{\text{Specific Activity (d/sec/meq)}}$$

in which the numerator is the body Na^{22} activity, and the denominator is the urine specific activity.

The changes in average per cent retention and total exchangeable sodium as a function of time for the 3 groups are shown in Fig. 3.

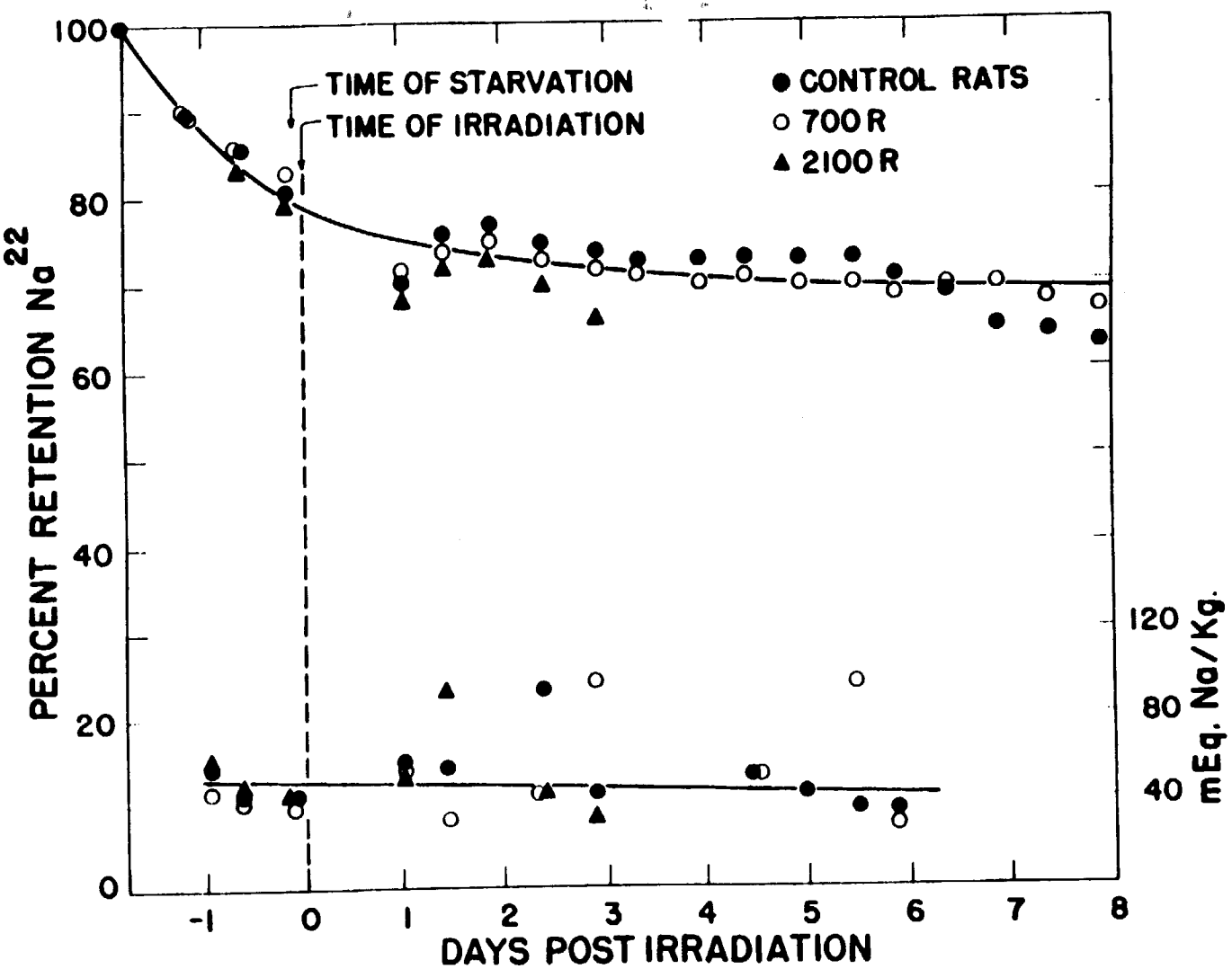

Fig. 3. Effect of 700 and 2100 rads of 250 KVP X-ray exposure on Na^{22} retention and total exchangeable sodium of fasted rats.

Discussion and Conclusions

The only significant feature of the data in Table 1, showing the effect of 2100 rads of irradiation on distribution of ingested sodium, is the fact that a major part of the dose of Na^{22} was still in the stomach 24 hours after exposure. This was not the case with animals fasted prior to irradiation. These observations indicate that gastric retention of food by heavily irradiated rats indirectly influences retention and absorption of orally administered Na^{22}. This is not a failure of the absorption process but rather a matter of physical obstruction in which the sodium is retained in the stomach along with the food.

The data in Fig. 1 definitely show that food deprivation produces a retention of body sodium and points out the necessity of comparing sodium excretion by heavily irradiated animals with fasted controls. When this was done (Fig. 2), there appeared to be no significant differences in the gross retention rate of Na^{22} by unirradiated rats and those receiving acute exposures of 1000 to 8000 rads of X irradiation.

An attempt to demonstrate an effect of 700 and 2100 rads of X irradiation on the total exchangeable sodium of fasting rats (Fig. 3) failed to show any significant differences

between irradiated groups and the control. The data scattered badly, perhaps because of difficulties in quantitative collection of 24-hour urine samples and inherent contamination with inert sodium during sample collection or during analysis with the flame photometer.

Although these studies are of a preliminary nature, they show rather conclusively that comparison of gross retention rates of Na^{22} in fasted control and irradiated animals fails to support the idea that increased sodium loss is either caused by radiation or plays a dominant role in the "intestinal death" syndrome of massive acute exposure.

References

(1) H. Quastler, Rad. Res. 4, 303 (1956).

(2) M. N. Swift and S. T. Taketa, Am. J. Phys, 185, 85 (1956).

(3) J. W. Osborne, Rad. Res. 4, 541 (1956).

(4) R. A. Conard, E. P. Cronkite, G. Brecher, and C. P. A. Strome, J. Appl. Physiol. 9, 227 (1956).

(5) G. Brecher and E. P. Cronkite, Personal communications quoted by Quastler in Ref. 1.

(6) E. A. Pinson, Physiol. Revs. 32(2), 123 (1952).

(7) F. D. Moore, J. D. McMurrey, H. V. Parker, and I. C. Magnus, Metab. 5(4), 447 (1956).

(8) K. T. Woodward, C. R. Richmond, and W. Langham, Proceedings Health Physics Society Meeting, p. 79 (1956).

Additional Observations on Electrolyte and Water Loss in Radiation Damage: Potassium^{42} and HTO

(C. C. Lushbaugh, D. B. Hale, and T. T. Trujillo)

Introduction

A recent study (1) using whole-body counting techniques revealed that, contrary to expectation (2), the heavily X irradiated rat did not lose sodium ion from extracellular tissues faster than a starved normal animal. Rats with morphologic damage typical of the "intestinal radiation death syndrome" conserved sodium ion to the same degree as normal starving animals. Although these experiments seemed to indicate that sodium loss was not the primary cause of death in this syndrome, it was thought possible that potassium or water, if lost, might produce changes in the intra- and extracellular ratios of potassium and sodium which would be incompatible with life. Preliminary experiments have now been completed in which the possibility of potassium and water loss was investigated in normal, starved, and irradiated rats.

Methods

Nine rats were given 56.5 μc K^{42} by stomach tube. Three were fed ad libitum throughout the experiment, and the other 6 animals were deprived of food but not water for 3 days before the isotope was administered. Twelve hours after isotope

administration, 3 of the 6 starved animals were irradiated with 2100 rads from a 250 KVP Maxitron unit. The dose was chosen because it was well within the range required to produce the so-called "gut syndrome of acute radiation death." A 2 x 2 in. sodium iodide crystal, in conjunction with a variable slit analyzer and rate meter, was used to count the animals during the early phase of the experiment, since the K^{42} gamma activity was too high for the liquid scintillometer. As the radiation decayed (12.5 hour half-life), the crystal was moved progressively closer to the rat. All animals were counted under identical conditions and all counts related to "standard," which consisted of another rat killed after oral administration of 5.6 μc K^{42} and kept refrigerated throughout the experiment. After 28 hours, the K^{42} count rate had subsided sufficiently to allow the animals and standard to be assayed in the Los Alamos small animal counter. Measurements were carried out immediately and at 11, 24, 30, 48, 54, 70, 80, 95, and 120 hours after administration of the isotope.

Another group was used to determine the amount of water lost following 2100 rads of acute X irradiation. One hour, 24, 48, and 72 hours after irradiation, groups of 3 rats were given 2.923 μc HTO in 1 ml of H_2O intraperitoneally. Three control animals received tritium water but no irradiation. Three and one-half to 4 hours later (approximate equilibration

time), the animals were killed after withdrawing 5 to 10 ml of blood by heart puncture. The tritium activity per ml of body water was determined by vacuum distillation of water from whole blood and radioassay of 0.5 ml of the distillate in an internal beta ray liquid scintillometer.

Results and Discussion

Figure 1 shows the exponential rate of loss of K^{42} by the normal, starved, and irradiated groups in the first experiment. Table 1 summarizes the per cent retention of the original tracer dose by the 3 groups at 95 hours after irradiation, at which time the irradiated group was moribund.

TABLE 1. PERCENTAGE OF K^{42} RETAINED AFTER 95 HOURS BY NORMAL, STARVED, AND IRRADIATED RATS

Rat	Normal	Starved	2100 rads
1	53.8	75.4	85.2
2	52.4	92.5	82.9
3	56.2	87.4	89.5
Average	54.1	85.1	85.9

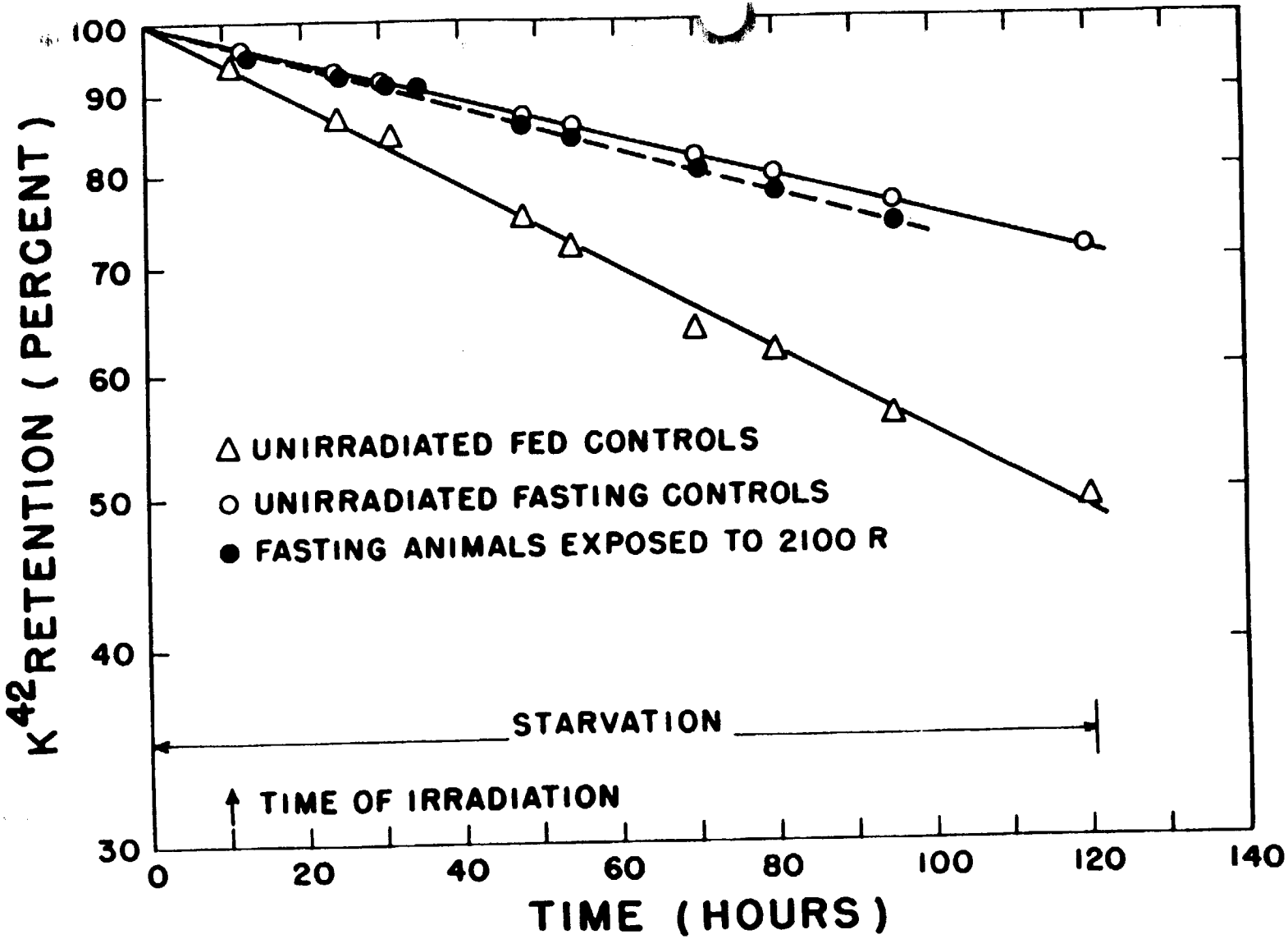

Fig. 1. Exponential rates of potassium loss in normal, starved, and irradiated rats.

These data failed to show any significant potassium loss in the irradiated animals. Their rate of potassium loss was slower than normally fed controls but essentially the same as that of unirradiated animals that were deprived of food. Loss in body weight and changes in total and per cent body water are contrasted in Table 2. In the 4 days preceding death from 2100 rads, the rats averaged about 20 per cent loss in body weight without any discernible loss in per cent of body water. In order for body weight to have remained constant at about 70 per cent, 14 per cent of the loss in body weight must have been water, while 6 per cent of the loss (3 g in a 250-g rat) was protein and fat.

These experiments appear to agree with previous findings (1), which implied that electrolyte (specifically, sodium) is not lost at an abnormal rate following massive acute radiation exposure. The data indicate that potassium, like sodium, is conserved in the starving irradiated rat and that any water lost is proportional to loss of protoplasm. These observations would appear to cast additional doubt on the validity of the concept of electrolyte and water loss being fundamental to the intestinal death syndrome following acute radiation exposure.

REFERENCES

(1) C. C. Lushbaugh, J. Sutton, and C. R. Richmond. The Question of Sodium Loss in the Intestinal Death Syndrome of Radiation Damage. This report.

(2) H. Quastler, Rad. Res. 4, 303 (1956).

TABLE 2. CHANGE IN BODY WEIGHT AND BODY WATER OF RATS FOLLOWING 2100 RADS OF X IRRADIATION

Body Weight			Body Water		
Initial (g)	Final (g)	Loss (g)	Tritium Activity (mc/ml)	Total (ml)	Per Cent
			No irradiation		
275	275	0	14.74	198.3	72.1
254	254	0	16.59	176.5	69.5
293	293	0	14.49	201.7	68.8
Average					70.1
			1 hour after irradiation		
239	239	0	17.35	168.5	70.5
255	255	0	16.66	175.5	68.8
212	212	0	18.08	161.7	76.3
Average					71.9
			24 hours after irradiation		
266	253	13	16.13	181.2	71.6
245	227	18	19.02	153.7	67.8
246	237	9	17.53	166.7	70.3
Average					69.9
			48 hours after irradiation		
238	220	18	19.65	148.8	67.6
260	240	20	17.89	163.4	68.1
232	213	19	19.58	149.3	70.1
Average					68.6
			72 hours after irradiation		
236	185	51	22.61	129.3	69.9
256	190	66	died before 4-hour equilibration		
238	203	35	died before 4-hour equilibration		
Average					69.9

A Stain Modification for Precise Identification of Three Connective Tissues (G. L. Humason and C. C. Lushbaugh)

Introduction

The staining method of Lewis and Jones (1) has been modified to combine ammoniacal silver carbonate, orcein in acid alcohol, and aniline blue following phosphomolybdic acid. Elastin, reticulum, and collagen can be selectively demonstrated on the same slide with exceptional clarity. It has found practical application in problems of pathology which pertain to vascular invasion and capsular infiltration by carcinoma, and degenerative diseases of connective tissues and blood vessels.

Results

Figure 1 shows photomicrographs (240x) of the staining characteristics as seen in an infiltrating mammary gland carcinoma (A) and, after addition of orange G to the procedure, in a thyroid carcinoma (B). These photomicrographs illustrate that the small, more easily invaded blood and lymphatic vessels are devoid of elastic tissue and are often best identified by the surrounding reticulum and collagen. This procedure makes such identification easier than by other, more common routine methods.

Reference

(1) A. L. Lewis and R. S. Jones, Stain Tech. 26, 85 (1951).

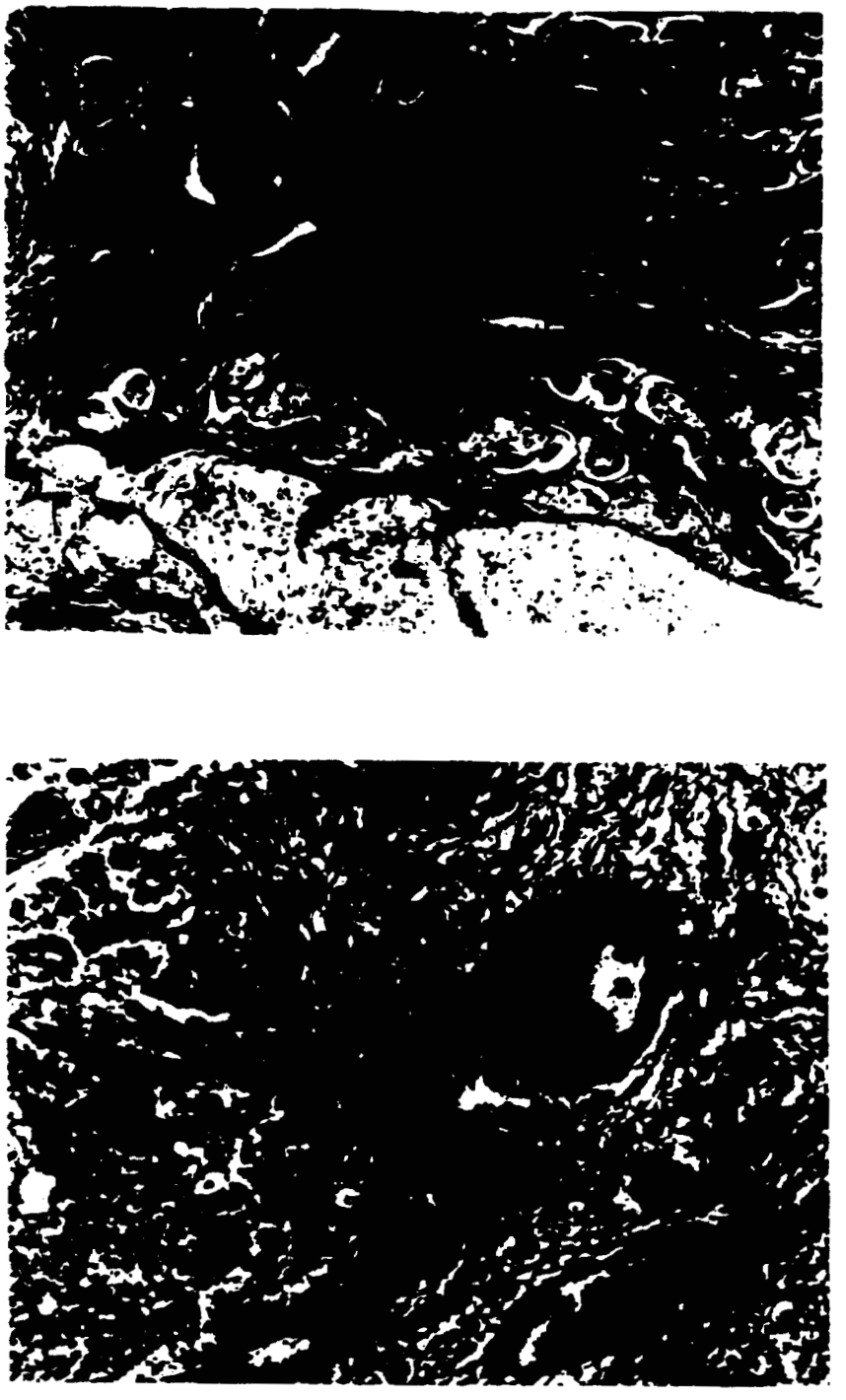

Fig. 1. Staining characteristics seen in an infiltrating mammary gland carcinoma (A) and in a thyroid carcinoma (B).

RADIOPATHOLOGY SECTION PUBLICATIONS

(1) C. C. Lushbaugh, H. Marvin, and D. B. Hale, The Life-span of the Radiochromated Erythrocyte, Abstract No. 1935, Fed. Proc. 18, Part I (1959).

(2) C. C. Lushbaugh and D. B. Hale, Whole-body Counting in Clinical Diagnosis, J. Nuc. Med., Sixth Annual Meeting Edition, Abstract (1959), p. 27.

MANUSCRIPT SUBMITTED AND ACCEPTED

(1) C. C. Lushbaugh, J. Sutton, and C. R. Richmond, The Question of Electrolyte Loss in the Intestinal Death Syndrome of Radiation Damage, Rad. Res.

CHAPTER 7

VETERINARY SECTION

Responsibility and Function

The Veterinary Section does no research per se. It is entirely a service section with the responsibility of supplying and caring for all experimental animals used for biological and medical research by the other sections. It is also the section's responsibility to maintain proper healthful conditions and adequate production and use records for the entire animal facility.

Stock supplies of mice, rats, guinea pigs, rabbits, dogs, and monkeys are maintained either by breeding or by purchase. Supervision, maintenance, and care of the stock animals are solely the responsibility of the Veterinary Section personnel. Once animals are placed on experiment, supervision of care and checking of the experimental results become the direct responsibility of the research staff member.

Routine cleaning, feeding, and watering of the experimental animals usually are performed by the caretaker personnel under joint supervision of the Veterinary Section Leader and the experimenter. Routine cleaning, feeding, and watering are maintained on a 7-day week basis.

Facilities

At the end of this report period, the animal facilities included 2 mouse breeding rooms; 1 mouse stock room; 1 dog room and runs; 1 stock rat room; 1 miscellaneous animal room; 1 wash room; 1 equipment room; 1 feed room; 1 kitchen; and 1 office. There is storage for equipment in the sub-basement. The experimental rooms are located on the laboratory floors and consist of 4 large rooms (12 x 24 ft) and 6 smaller rooms (8 x 24 ft).

An addition to the animal facilities is under construction at a cost of approximately $325,000. The new facilities include 5 monkey pens with outside exercise areas (maximum capacity 50 animals); 19 dog kennels with outside runs (maximum capacity 57 animals); 13 mouse rooms (maximum capacity 40,000 animals); mouse feeding room; dog and monkey feeding room; dog breeding room; monkey metabolism room; dog metabolism room; large animal quarantine room; autopsy and treatment area; storage area for veterinary supplies; veterinary office; mouse

cage cleaning room; mouse bedding and cage storage area; and locker room for animal caretakers. The addition will be completed in March 1960.

Animal Stocks

Mice.--All mice used by the Biomedical Research Group are bred and raised in the animal facility. No purchases are made from commercial suppliers. Usually only females are used in experiments. The basic mouse for the production of the laboratory stock is the RFM purebred strain (brother-sister mated) whose offspring are randomly mated to provide a RF strain which is used exclusively in routine mouse experiments. The breeding facilities thus produce all the stock breeding mice, as well as its own breeder stock replacements. A purebred AKR albino strain is maintained and used exclusively for special studies. Production is based upon requested numbers. A randomly mated CFW strain is bred and carried in the mouse colony primarily for special studies and correlations with past experiments conducted at a time when the CFW mouse (obtained commercially) was the standard animal used in this laboratory. Production is based on the number requested.

Rats.--The only rats used in the laboratory are of the Sprague-Dawley strain. These are supplied by the commercial

supplier. Male rats are received in biweekly shipments of 100 animals per shipment. Females are obtained occasionally on special order.

Dogs.--The only dogs used in the laboratory are pure-bred beagles that are bred and raised in the animal facility. The parent stock was obtained in 1954 from the beagle colony of the University of Utah project.

Monkeys.--Monkeys have been used in the past only for special experiments. Stocks have been obtained through the collaboration of the USAF School of Aviation Medicine at Brooks Air Force Base. Very few animals have been kept on hand because of inadequacy of facilities. The strain used is Macaca mulatta. In the future, increased experimentation with monkeys is anticipated in connection with the investigations of interspecies correlations in the metabolism of radioisotopes (see Biochemistry Section).

Rabbits and Guinea Pigs.--Rabbits and guinea pigs are obtained only on request of the experimenters and are ordered from a carefully selected commercial supplier. The demand is not great. Rabbits are being supplied by Bunny Run of La Puente, California; and guinea pigs are obtained from Adams Cavary of San Gabriel, California.

Animal Inventory (1959)

For convenience, the animal inventory is given for the entire calendar year of 1959 and is as follows:

Mice

RFM Strain

Total number babies born	4,053
Total number weanlings	3,417
Weaning percentage	84.29
Number breeding females	143

RF Strain

Total number of female weanlings	12,147
Number of females delivered for experimentation	8,586
Number of females in stock	4,890
Number of above being held for aging studies	1,150
Number of breeding females	1,312

AKR Strain

Total number babies born	3,038
Total number weanlings	2,271
Weaning percentage	74.75
Number delivered for experimentation	
females	1,183
males	350
Number of breeding females	126

CFW Strain

Total number of female weanlings	1,631
Number of females delivered for experimentation	1,631
Number of breeding females	54

Sprague-Dawley Rats

Total number received	2,923
Number delivered for experimentation	1,328
Number male rats in stock	817
Number of above being held for aging studies	377

Beagles

Number bred	0
Number male beagles for breeding and stock	27
Number female beagles for breeding and stock	9

Monkeys

Number in stock (males only)	3

Rabbits and Guinea Pigs

Number of rabbits received	36
Number in stock	18
Number of guinea pigs received	24
Number in stock	10