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Biology and Medicine
(TID-4500, 13th Ed.)

BIOLOGY RESEARCH ANNUAL REPORT

1956

By

The Staff of the
Biology Operation

H. A. Kornberg, Manager

January 4, 1957

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INTRODUCTION

H. A. Kornberg

This volume is the sixth in a series that annually summarizes the research activities of the Biology Operation. The previous volumes in this series and the years reported are: HW-25021 (1951), HW-28636 (1952), HW-30437 (1953), HW-35917 (1954), and HW-41500 (1955).

Late in 1956 a reorganization at Hanford disbanded the Radiological Sciences Department and transferred the Biology Section, with its name changed to Biology Operation, to the Hanford Laboratories Operation.

Also during the year the research programs continued to change from being primarily Hanford oriented toward being mostly concerned with radiobiological problems derived from the general needs of radiation hazard control. Some of this laboratory's results follow.

Evidence, obtained in 1955, was confirmed that ingesting P^{32} is not absorbed and deposited on the skeleton to the extent currently used in establishing permissible limits. However, there appears to be a marked effect of age of the experimental animal on absorption and deposition. This effect was found to occur with ruthenium and plutonium also.

The effect of calcium on skeletal deposition of strontium was again measured for an acute dose, administered either intragastrically or intraperitoneally. The strontium deposition did not decrease as the calcium was increased. Radiophosphorus in the root environment of plants gave similar results. Isotopic dilution by non-radioactive phosphorus did not decrease the uptake of P^{32}.

Following ingestion of Ru^{106} by rats, the greatest early radiation dose occurred in the large intestine. Chronic feeding of Ru^{106} indicated that three organs almost equally qualify as being the critical ones - the kidney, the bone, and the gonads. Permissible limits for Ru^{106} in drinking water are derived on these bases.

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Also regarding maximum concentrations was work with Cs^{137}, which essentially reconfirmed the presently accepted figure. On the other hand, research on the sensitivity of the gastrointestinal tract of the rat indicated that the tract cannot be considered a critical organ for alpha emitters, but that it is reasonably sensitive to the presence of poorly absorbed beta emitters.

Continued work with I^{131} fed daily to sheep showed that 5 μc/day definitely increases the incidence of thyroid adenomas and decreases protein-bound iodine. A decreased thyroidal uptake of I^{131} was noted at 0.5 μc/day. Pigs that daily received a smaller amount of food than normally needed were observed to deposit less of the daily quantity of I^{131} fed them than did pigs on a normal diet. However, there was no difference in blood constituents between pigs on the normal and restricted diets.

Other work with large animals included the development of a special metabolism cage for pigs receiving isotopes, determination of effects of a triple radiation assault to lambs, and defining the sequela of beta skin burns on pigs.

The skin absorption of plutonium from a PuF_4 solution was found to be similar to that of $Pu(NO_3)_4$. Animal skin contaminated with plutonium was effectively decontaminated by applying a plastic coating to the contaminated area and then peeling it off.

In animals injected with radioactive particles, a rapid initial removal from the lung was noted followed by increasingly slow removal. Chemical carcinogens were found to not act synergistically with radioactive particles or external radiation in producing lung cancers in mice.

CaEDTA was observed to increase the sensitivity of yeast to radiation from plutonium or tritium. There was no effect at high irradiation dose rates. Plutonium was also observed to exert a toxic chemical effect on yeast by inhibiting glucose uptake and respiration.

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The uptake of Sr^{90} into plants from contaminated soil was seen to increase with time. Each growing season apparently increased the availability of radiostrontium. When radiostrontium-contaminated plants or inorganic solutions were fed to sheep, four per cent of the daily dose appeared in milk.

These remarks may serve to highlight some of the papers contained in this report. Other papers relate to the topics outlined and to measurements of the effects of production on animals in the environment of the Hanford Works.

This year I am indebted to Donald E. Warner for his assistance in editing this report and to Miss B. Jane Peterson for the co-ordination and preparation of much of the manuscript. Authors of papers wish to express their appreciation for the analytical services performed for them by A. C. Case and personnel of the Radiochemistry Laboratory.

1086572

EARLY FATE OF INGESTED RUTHENIUM

R. C. Thompson and O. L. Hollis*

The distribution of Ru^{106} within the various segments of the gastrointestinal tract of the rat was determined at intervals from 35 minutes to 30 hours following intragastric administration. From the data obtained the largest radiation dose was estimated to be received by the lower large intestine, with the doses to other segments being lower by as much as a factor of five. There appeared to be no significant holdup of the radioisotope due to absorption on the intestinal wall. Judging from the rate of clearance of Ru^{106} from the blood, significant absorption from the gastrointestinal tract occurs during not more than the first 35 minutes following intragastric administration. Maximum concentration of Ru^{106} in the kidneys is attained within three hours, and a major fraction of this kidney ruthenium is lost with a half-life of about one day.

This experiment involving the intragastric administration of Ru^{106} to 72 female, Sprague-Dawley rats, was described in the previous Annual Report (1). The complete results were reported in detail in a separate document (2). Some of the more significant aspects of these results are reviewed below.

RESULTS AND DISCUSSION

Examples of the pattern of Ru^{106} distribution along the gastrointestinal tract of individual rats at various time intervals following intragastric administration are shown in Figure 1. Figure 2 shows a photograph and autoradiograph of the tract from the 125-minute animal of Figure 1. The total distribution data indicate quite clearly that the Ru^{106} is not appreciably adsorbed to the intestinal wall but moves along in association with the food residues in the tract.

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*Present Address: Dow Chemical Company, Freeport Texas.

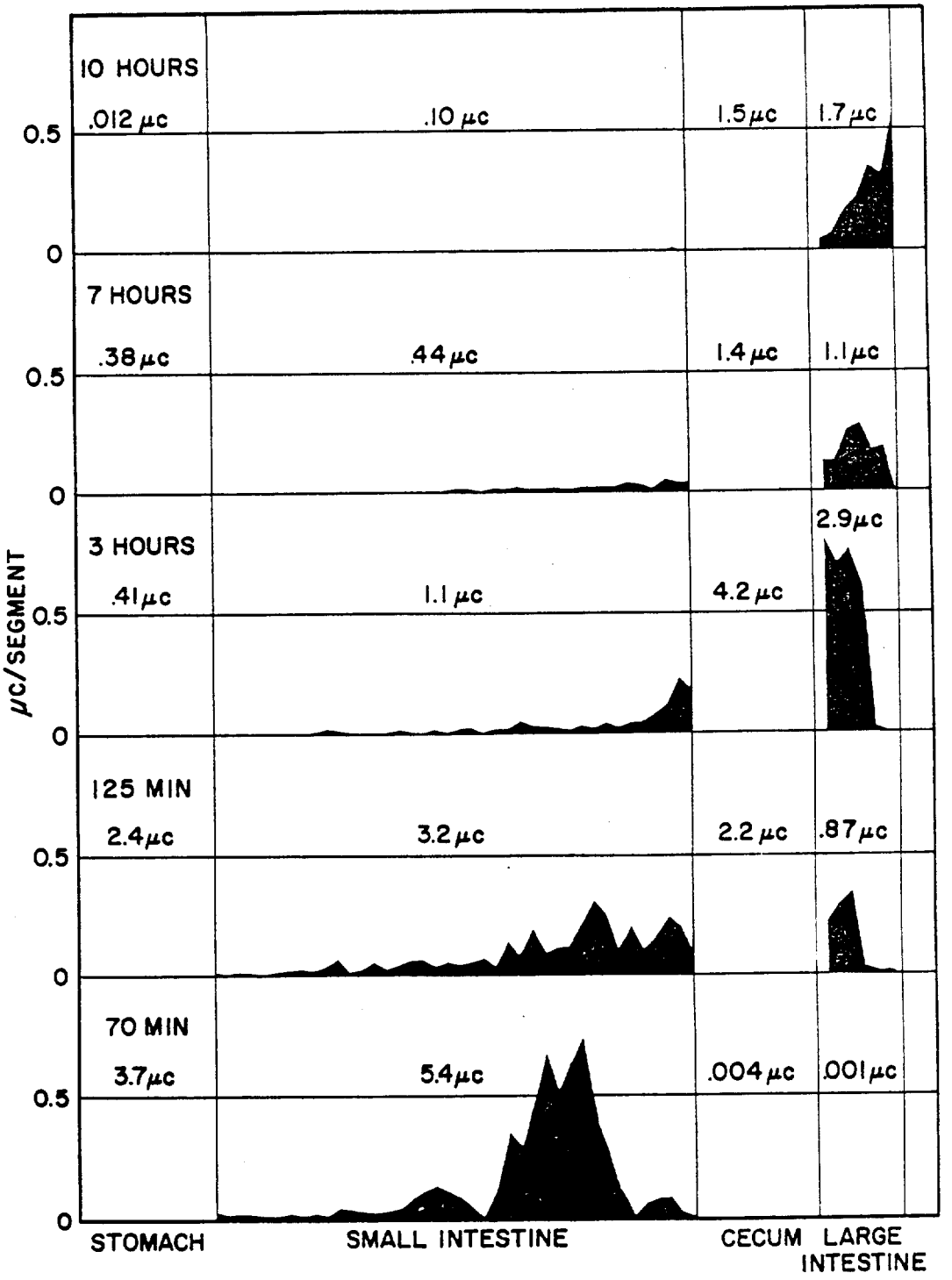

FIGURE 1

Distribution of Intragastrically Administered Ru^{106} within the Gastrointestinal Tract at Various Time Intervals Following Adminstration.

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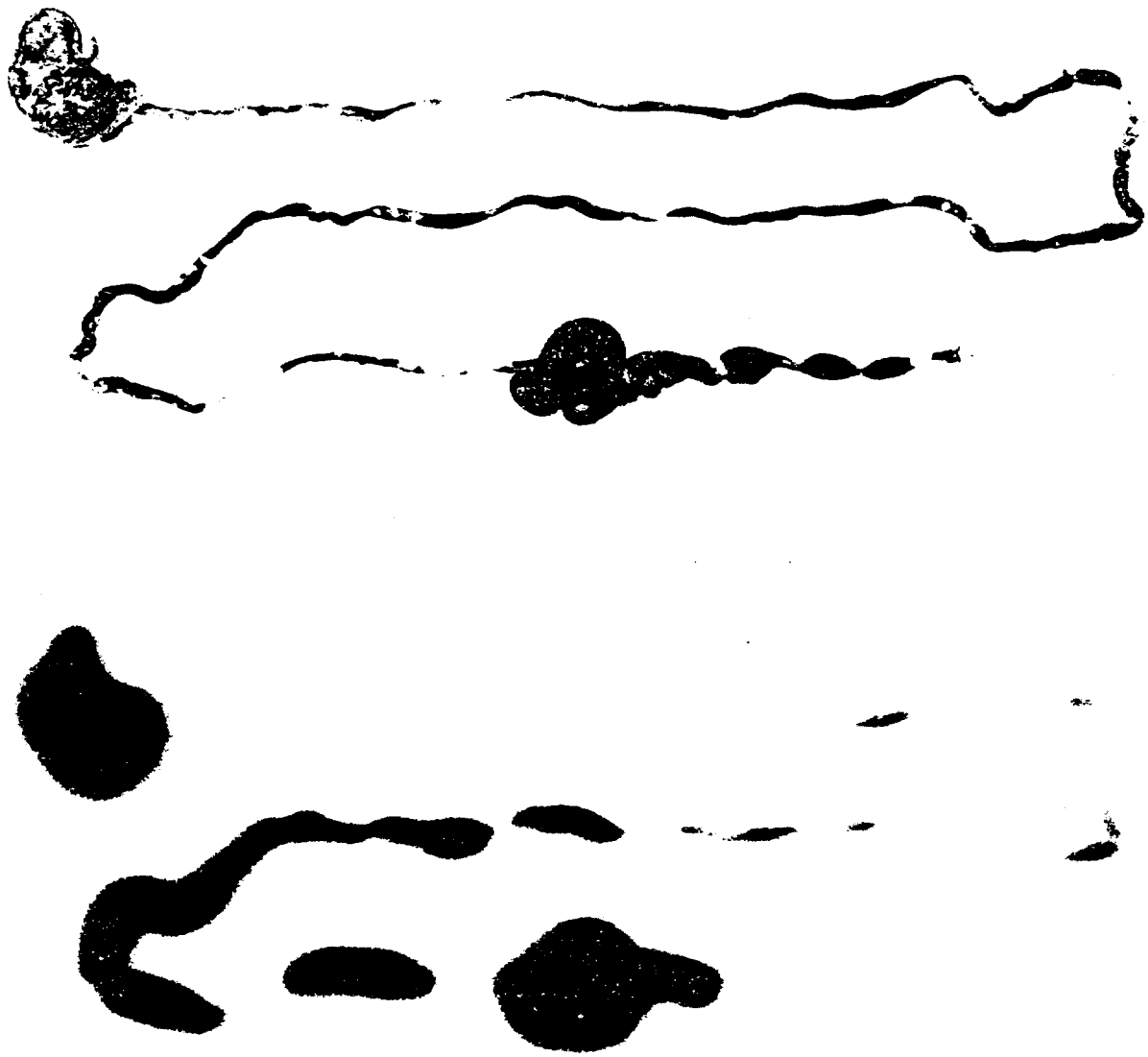

FIGURE 2

Photograph and Autoradiograph of Gastrointestinal Tract Removed 125 Minutes After Intragastric Administration of Ru^{106}

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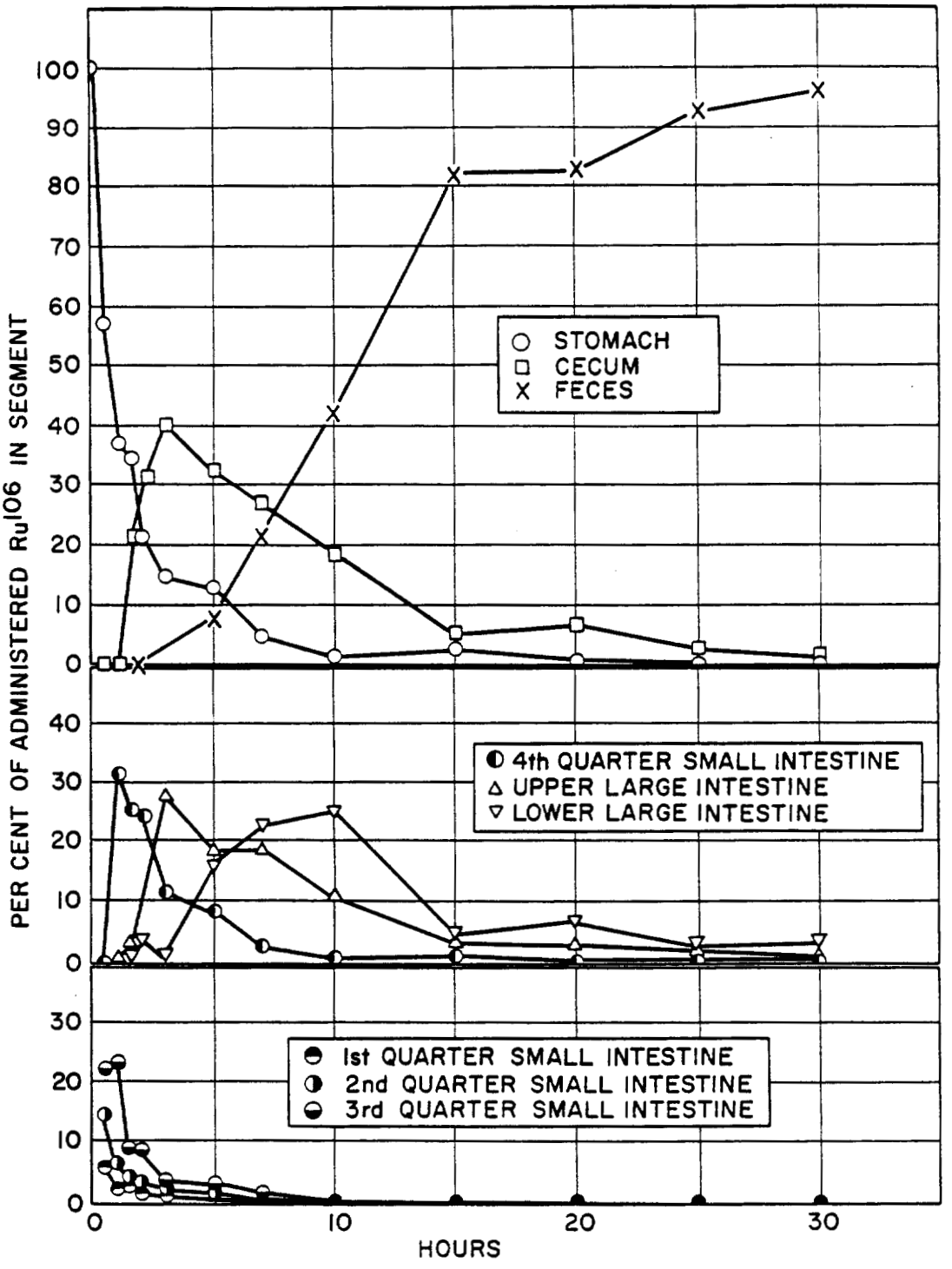

FIGURE 3
Distribution of Ru^{106} Along the Gastrointestinal Tract at Intervals Following Intragastric Administration.

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TABLE 1

Irradiation of Various Segments of Gastrointestinal Tract of Rats by Intragastrically Administered Ru^{106}

Segment of gastro-intestinal tract	Time integral of Ru^{106} concentration*	Dose in rad from 8 μc Ru^{106}
Stomach	.38	4.5
Small intestine		
1st quarter	.45	5.4
2nd quarter	.36	4.3
3rd quarter	.50	6.0
4th quarter	.61	7.3
Cecum	1.0	12
Large intestine		
1st half	1.1	13
2nd half	2.0	24

*Units of fraction of administered dose times hours divided by mass of segment contents in grams.

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Ruthenium-106 retention in blood and kidneys is shown in Figure 4. Each point is the average of analyses on 6 rats. From the steep drop in blood Ru^{106} during the interval from 35 to 70 minutes, it is evident that absorption was largely completed during the first 35 minutes following feeding; this despite the fact that at 35 minutes post-feeding over half of the ruthenium was still in the stomach. Apparently ruthenium is converted in the gastrointestinal tract to a non-absorbable state, soon after ingestion. Total absorption in the present experiment was higher than the 3 per cent absorption measured in previous chronic feeding studies (3). Blood and kidneys alone account for nearly 5 per cent of the administered dose, 35 minutes after feeding. The higher absorption may be due to an 18-hour fasting period, prior to intragastric administration, which was employed in the present experiment, but not in earlier studies.

The majority of absorbed ruthenium was rapidly cleared from the blood. The half-life of this process, as indicated by the steep early portion of the blood curve, is not longer than 30 minutes. The latter portion of the blood retention curve exhibits a biological half-life of the order of one day. After the initial buildup in the kidneys, ruthenium is lost with an apparent half-life of about one day. Kidney ruthenium components with much longer half-lives are known to exist from previous retention studies of longer duration (3).

ACKNOWLEDGEMENTS

We wish to acknowledge the technical assistance of Alma Crosby.

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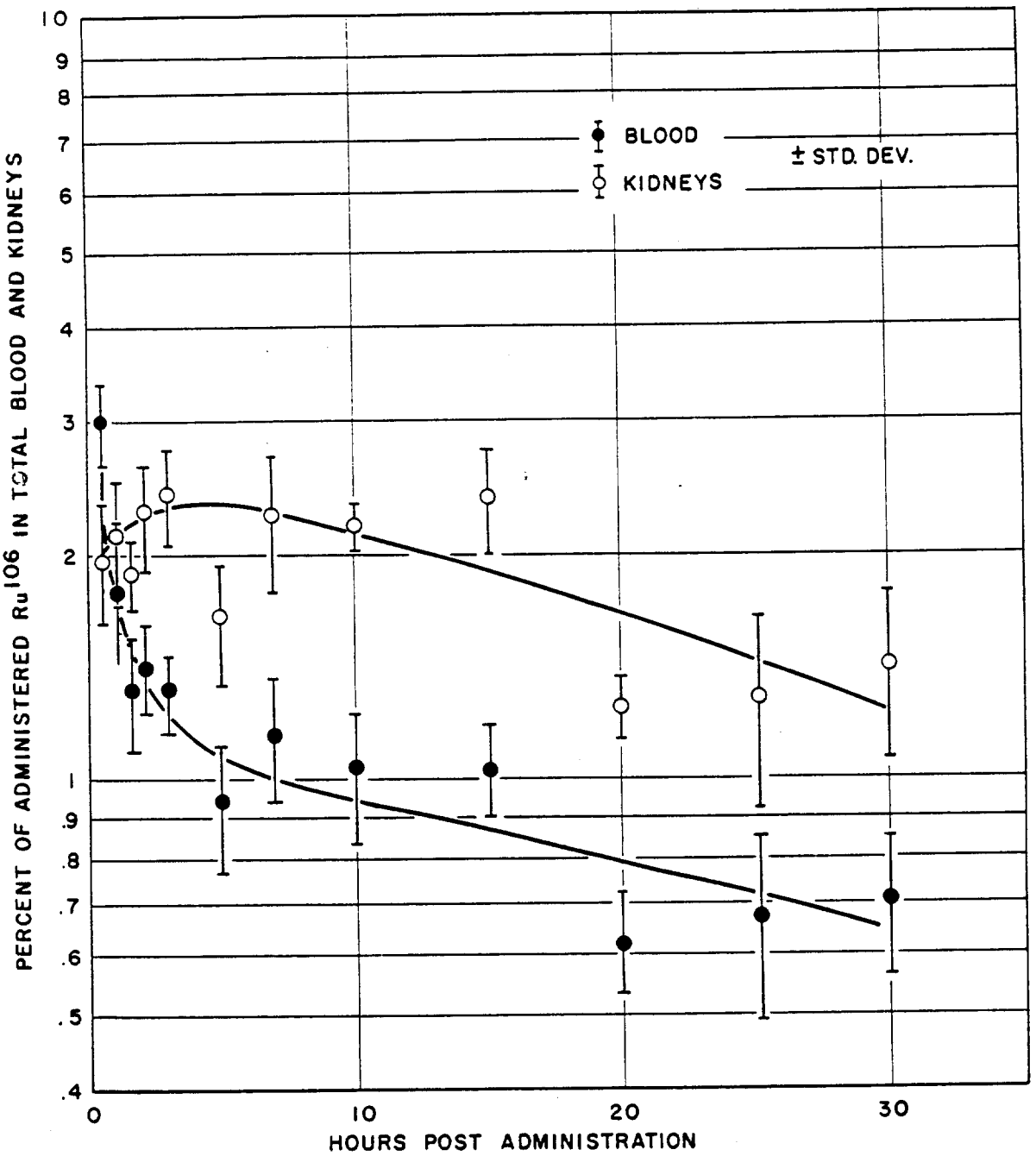

FIGURE 4
Early Retention of Ru^{106} in Blood and Kidneys Following Intragastric Administration

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DISTRIBUTION AND RETENTION OF RUTHENIUM

J. E. Ballou and R. C. Thompson

Chronic exposure of rats to Ru^{106} in their drinking water for periods of up to 500 days resulted in tissue ruthenium concentrations of comparable hazard concern in kidneys, bone, and gonads. A maximum permissible concentration for Ru^{106} in water of 4×10^{-3} μc/ml would be approximately correct on the assumption of any of these three tissues as the "critical organ".

In a continuation of studies reported in the previous Annual Report (1, 2), chronic exposure experiments involving both mature and immature rats have been performed. The results from these experiments permit a direct evaluation of permissible exposure limits for the rat, without assumptions as to fraction absorbed, biological half-life, etc. The difficulties which remain are principally those involved in the extrapolation of rat results to man. A complete account of these experiments and their hazard implications appears in a separate document (3).

METHODS

In the principal experiment, 60 male rats and 60 female rats were maintained on drinking water containing 0.01 μc Ru^{106}/ml. Groups of animals were killed after 50, 125, 200, 300, 400, and 500 days, and the Ru^{106} content of organs and tissues determined. In a second experiment eleven male and eleven female rats were placed on a similar drinking water regimen at weaning. These animals were killed in two groups after 77 and 150 days exposure.

The Ru^{106} employed was the "carrier-free chlorides" (specific activity 4 to 10 c/g) obtained from Oak Ridge, diluted and adjusted with NaOH to a pH of 2.0. Water consumption was estimated by weighing the water bottle before and after each filling. Details of the sampling and radioassay procedures have been previously described (2).

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RESULTS AND DISCUSSION

Results for the tissues of principal hazard concern are shown in Table 1, expressed as a fraction of daily ruthenium intake present in these tissues after various periods of chronic feeding. It will be appreciated that some uncertainty is inherent in these figures due to the lack of consistency in the daily intake. This is especially true in the case of the animals exposed from weaning.

For all feeding periods, the concentration of ruthenium attained in the kidney was higher than that in any other tissue by at least a factor of two. The kidney to bone ruthenium concentration ratio was greater than 5 in the animals exposed after reaching maturity. In the animals exposed from weaning this ratio was 3.6 for males and 3.3 for females after 77 days' exposure. On the conventional assumption that a radioisotope deposited in bone is 5 times as hazardous as the same average concentration of the radioisotope in any other tissue (4), bone should be considered the critical organ for the immature rat, and kidney the critical organ for the mature rat.

The data of Table 1, together with the calculated permissible critical organ burden (to deliver 300 mrad per week) leads directly to an evaluation of the maximum permissible daily intake, and thus to the maximum permissible concentration (MPC) in water (2). In applying such calculations to the human it is conventionally assumed that the fraction of radioisotope intake deposited in the critical organ of man will be the same as that deposited in the same organ of the experimental animal. On such a basis MPC's calculated for various assumed critical organs in the human are as follows:

Critical Organ	MPC ($\mu c/ml$)
Kidney	5×10^{-3}
Bone	13×10^{-3}
Testes	4×10^{-3}
Ovaries	11×10^{-3}

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TABLE 1

Fraction of Daily Ruthenium Intake Present in Kidney, Bone And Gonads after Various Periods of Chronic Feeding

Experiment	Duration of Feeding (d)	Kidney		Bone		Gonads	
		Male	Female	Male	Female	Male	Female
Animals fed after attaining maturity	50	.010	.014	.014	.013	.0014	.00020
	125	.015	.017	.021	.016	.0025	.00020
	200	.011	.015	.014	.017	.0024	.00019
	300	.009	.010	.019	.018	.0032	.00016
	400	.011	.014	.014	.019	.0030	.00019
	500		.014		.021		.00015
Animals fed from weaning	77	.011	.012	.028	.037	.0011	.00013
	150	.007	.009	.019	.021	.0013	.00012
Value used in calculations		.015		.030		.003	.0002

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On the basis of these calculations, testes are the critical organs. Although testes accumulate a lower Ru^{106} concentration than either kidneys or ovaries in the rat, the extrapolation to man of "fraction of intake deposited per organ" results in a higher apparent concentration in human testes, because of the much smaller mass of testes relative to body size in man as compared to the rat. For similar reasons these calculations result in bone appearing less critical than kidney in man, whereas it is more critical than kidney in the immature rat.

In view of the difficulties involved in extrapolating from rat to man, the unequivocal choice of a critical organ is impossible. Considering all of the data, a value of 4×10^{-3} $\mu c/ml$ for the MPC in water would appear to be a reasonable choice. This is the value previously recommended on the basis of incomplete results from these studies (1, 2), and compares with the present officially proposed value of 0.1 $\mu c/ml$ (4).

ACKNOWLEDGEMENTS

We wish to acknowledge the technical assistance of Joan Hess and Margaret Lawson.

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1086585

EFFECT OF AGE ON THE ABSORPTION OF PLUTONIUM AND RUTHENIUM

J. E. Ballou

Gastrointestinal absorption of plutonium and ruthenium by immature rats exceeded adult absorption by as much as 85 times for plutonium and 5 times for ruthenium. Plutonium distribution was essentially the same in the seven-day-old rat and the adult. Ruthenium distribution varied considerably with age, but at all ages the highest concentrations were found in kidney, liver, femur and ovaries.

Increased deposition of Ru^{106} in the bone of young rats has been described in a recent report from this laboratory (1). These findings had significant effects on the choice of critical organ and subsequent calculation of an MPC for Ru^{106}. Further studies extending these observations are presented here in which the gastrointestinal absorption and distribution of plutonium and ruthenium were determined in rats of several age groups.

METHODS

The animals fed plutonium received 0.05 ml of a pH 2 solution containing approximately 100 μg of Pu (IV). Those receiving ruthenium were fed 0.05 ml of a pH 2 solution containing approximately 3 μc of Ru^{106}. The very young rats were fed using a plastic tube of about the diameter of a 27-gauge needle attached to a 1/4 ml syringe. At least five and usually more rats were fed at each age level. Animals were sacrificed 21 days after exposure to plutonium or, in the case of ruthenium, after 24 hours.

Samples analyzed for plutonium included liver, lung, carcass and, in some animals, total bone and soft tissue. The ruthenium content of several tissues and organs was determined in the usual manner. Gross differences in the rate of gut clearance due possibly to age or radiation were determined by estimating the progress of the ruthenium through the gut with

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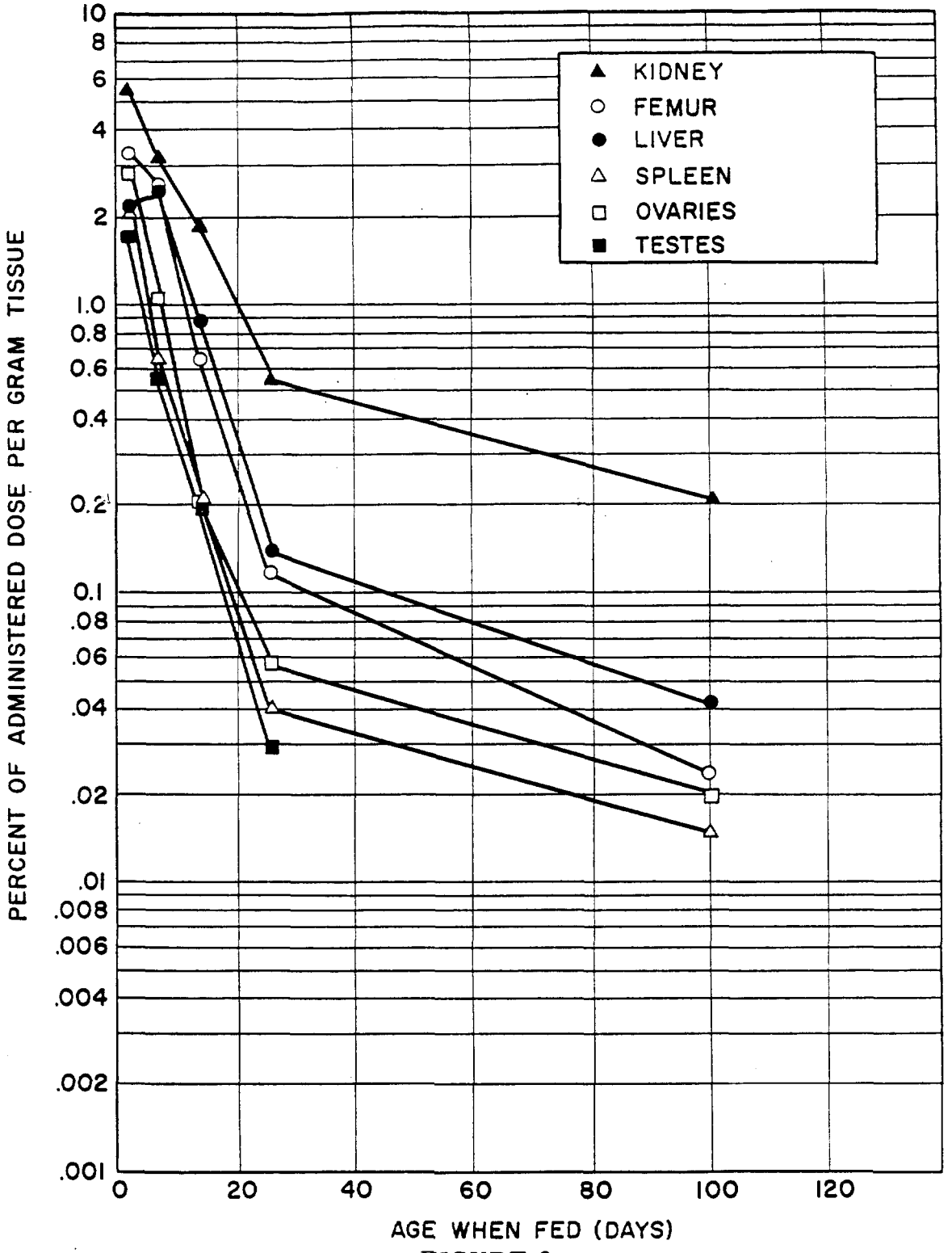

FIGURE 3

Effect of Age on the Distribution of Ruthenium

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DISTRIBUTION AND EXCRETION OF PLUTONIUM

B. Kawin, N. L. Dockum, and R. F. Palmer

Following intravenous injection, plutonium distribution in several cellular fractions and plasma components is being studied. In urine, plutonium may be associated with S^{35}, as suggested by paper chromatography. Tissue localization of plutonium is being studied by autoradiography. Preliminary results are reported at this time.

METHODS

Three hours after intraperitoneal injection of aqueous sodium sulfate abeled with 5 μc of S^{35}, two young adult female, Sprague-Dawley, rats were ntravenously injected with 50 μg of Pu^{239} (IV) as the citrate complex, pH 5. 'ive and sixty minutes later the rats were killed and the following techniques 'ere employed on tissues and fluids: paper electrophoresis of plasma pro- eins, ultracentrifugation (1) of liver, kidney, spleen, and muscle tissue nto cellular components, paper chromatography of accumulated bladder rine, using a solvent designed for separation of phosphates (2), and auto- adiographic determination of localized Pu^{239} in tissue.

RESULTS AND DISCUSSION

'lutonium Distribution in Plasma Proteins

Of the plutonium in the plasma from the rat killed 5 minutes after ıjection, more than 50 per cent was associated with the β-globulin fraction. ı the one-hour animal, however, the plutonium was distributed almost niformly between the β-, α_1-, and α_2-globulin fractions. Almost equiva- ent amounts of plutonium were associated with the albumin fractions in oth plasma samples. These results suggest that the distribution of plutonium ı plasma proteins may vary with time after injection.

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Plutonium Distribution in Cellular Components

The relative concentrations of plutonium in the nuclear, mitochondrial, and microsomal fractions of liver, kidney, spleen, and muscle one hour after intravenous injection are listed in Table 1. The soluble cell fractions contained less than 0.5 per cent of the total. The plutonium did not appear to concentrate selectively or functionally in the cellular fractions.

Plutonium Distribution in Urine

Following resolution of the urine samples by paper chromatography several peaks of plutonium radioactivity were detected. These were closely associated with maxima of S^{35} radioactivity measured on the same filter paper strips.

Plutonium Localization in Tissues

In the animals administered plutonium and killed five minutes and one hour later, the autoradiographic technique revealed that the concentrations of plutonium in the liver and the kidney were approximately the same at both time intervals. There was no apparent increase in the one-hour animal compared with the five-minute animal.

The concentration of plutonium in the spleen of the five-minute animal was less than that in the animal killed at one hour. The concentration in skeletal muscle was greater in the animal killed at five minutes than in the animal killed after one hour. There was a detectable concentration in the ovary of the animal killed at one hour but it was less than the track densities observed in the major organs of the same animal. Increased concentrations over areas containing blood or blood vessels were apparent in the animal killed at one hour.

Autoradiograms illustrating certain of these observations are shown in Figures 1 and 2.

1086589

TABLE 1

Relative Percentage of Pu^{239} in Cell Components of Rat One Hour After Intravenous Injection*

Fraction	Nuclear Fraction	Mitochondria	Microsomes
Liver	26	28	46
Kidney	27	41	32
Spleen	32	13	55
Muscle	27	42	30

*Fifty μg of Pu^{239} (IV) were injected in citrate buffer pH 5.

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FIGURE 1

Autoradiogram of Lung Tissue (one hour) Illustrating Low Alpha Track Density over Alveolar Area with an Increased Track Density over the Vascular Areas. (110X)

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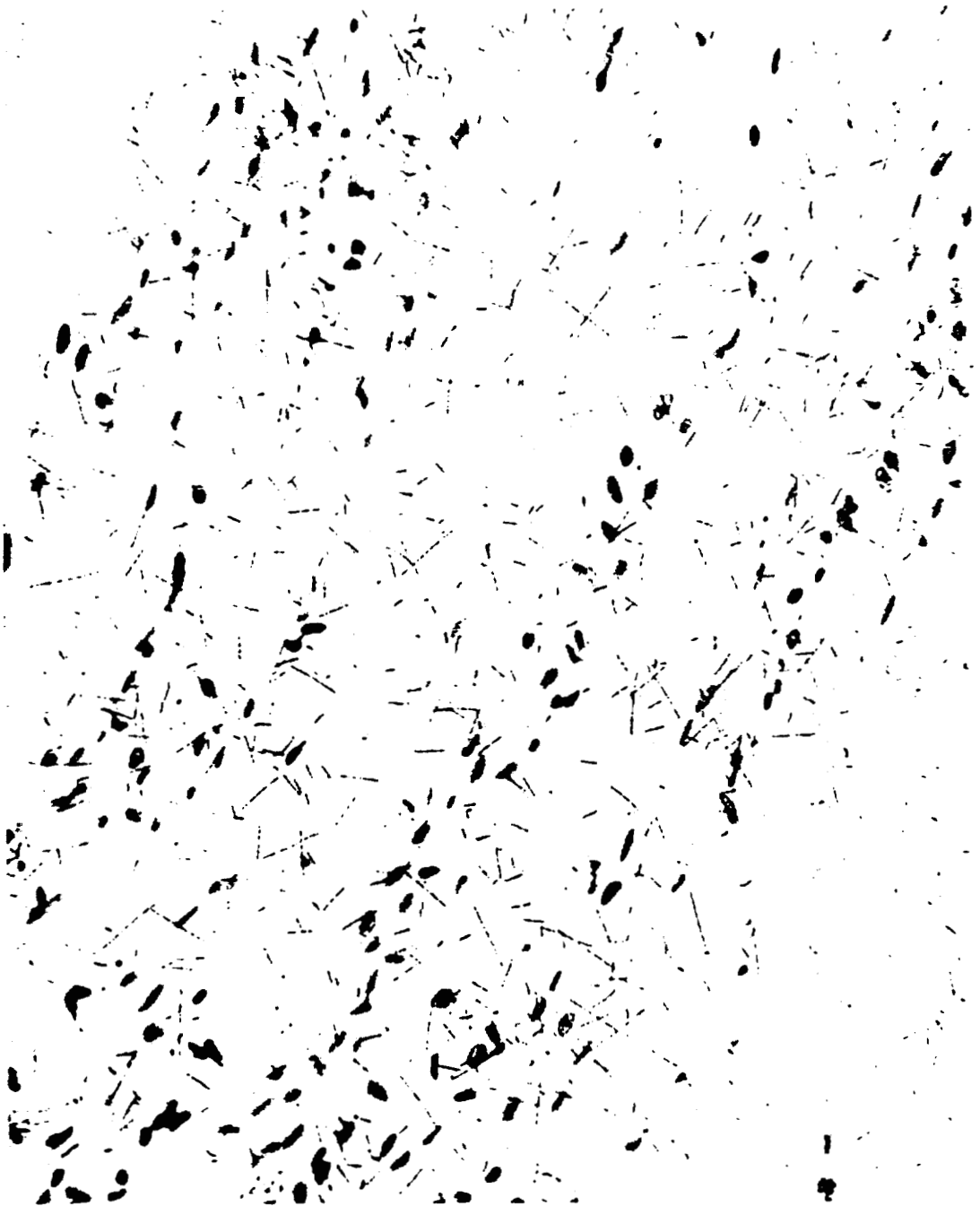

FIGURE 2

Autoradiogram of Longitudinal Section of Striated Muscle (one hour) Illustrating the Fairly Even Distribution of Alpha Tracks Above Muscle Fibers. (200X)

The preliminary results here reported indicate rapid and widespread distribution of intravenously injected plutonium in several fractions of rat plasma, tissues, and urine. Further studies are continuing to typify these fractions and to determine more closely the kinetic behavior of plutonium in the rat.

ACKNOWLEDGEMENT

The able technical assistance of D. S. DeChant, E. J. Coleman and G. Vogt is gratefully acknowledged.

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result of the incorporation of the electron dense element, Pu^{239}, into the collagen fibrils. The characteristics of the collagen in each of these micrographs are summarized in the "electronoscopic observations" column of Table 2.

Shrinkage temperature data are also summarized in Table 2. The T.S. of untreated cowhide collagen is 63 C. Treatments which lower the T.S. of collagen rupture stabilizing bonds or cohesive forces holding the collagen fibril together; T.S. 's higher than normal are an indication that stabilizing forces have been added to the collagen fibril.

The results in Table 2 indicate that prolonged soaking in water and nitric acid ruptures cross-linking bonds of collagen fibrils. The increase in hydrothermal stability of the salt-treated collagen is the result of weak salt links between the collagen polypeptide chains. The salt cross-linking is temporary, and can readily be removed by washing the collagen. Salt cross-linking might be considered an example of physical bonding. The shrinkage temperature of the acid plutonium treated collagen was difficult to determine because the aggregates of "collagen plutoniumate" were very small and transluscent. However, repeated T.S. determinations verified that the collagen plutoniumate did not shrink below 65 C. The high shrinkage temperature of the collagen from the reaction mixture, originally containing sodium chloride and plutonium, indicates that the plutonium forms stable chemical inter-chain bonds in the collagen fibrils.

The macro periodicity of the collagen plutoniumate is smaller than that of control collagen. This is the result of shrinkage caused by the reaction of collagen with plutonium. Such shrinkage is common when collagen is tanned to make leather. In fact, the fibrils shown in Figure 3 are plutonium-tanned leather. Thus, when collagen is reacted with plutonium *in vitro*, a stable chemical compound is formed.

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TABLE II

Summary of Electronoscopic Observations and T.S. Measurements of the Collagen from the Various Reaction Mixtures

Reaction mixture	Electronoscopic observations	T.S.	Remarks
Control (Figure 1)	Fibrils indefinitely long compared to width (approx. 0.1 μ). Macro-period = 650A (avg.) Intra-period bands, indistinct. Electron density low.	63 C	No dialysis
H_2O (75 hrs)	Fibrils break up into their component filaments (4)	55 C	No dialysis
0.2 N HNO_3	Fibrils completely filamented. (4)	23 C	No dialysis
10% NaCl	Same as control-above. Salt crystals.	75 C	No dialysis
	Same as control-above. No salt crystals.	63 C	NaCl removed by dialysis
0.2 N HNO_3, 0.2% Pu^{239} (Figure 2)	Fibrils swollen; electron dense back bone. Macro-period 400A (avg.) Intra-period striations indistinct.	>65 C	Acid removed by dialysis
10% NaCl, 0.2 N HNO_3, 0.02% Pu^{239} (Figure 3)	Fibrils indefinitely long compared to width - .075 μ (avg.) Macro-period = 480A (avg.) 6 intra-period dark striations	78 C	Salt and acid removed by dialysis

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1086596

DISTRIBUTION AND RETENTION OF CESIUM

J. E. Ballou

A maximum permissible concentration for Cs^{137} in water of 1.8×10^{-3} μc/ml was calculated from coefficients derived from the retention curve for Cs^{137} in muscle. This differs insignificantly from the presently accepted value of 2×10^{-3} μc/ml. In a chronic feeding experiment now in progress cesium concentrations have not yet reached equilibrium levels after 100 days' exposure to Cs^{137} in drinking water.

Previous studies (1) had suggested that cesium might be retained with a longer biological half-life than usually assumed, and that its rate of loss from tissues might change with time following exposure. The purpose of this experiment was to investigate these possibilities by determining radioactive cesium retention for long time periods following a single administration of the isotope. An additional experiment, extending work done earlier in this laboratory, is now in progress in which cesium absorption and distribution is being studied following long-term chronic exposure to Cs^{137} in the drinking water. A detailed account of the single administrative experiment is contained in Document HW-46150 (2).

METHOD

In the acute exposure experiment Cs^{137} was administered intraperitoneally to 35 female rats of the Sprague-Dawley strain, weighing 240 ± 25 grams. Each animal received a single injection of 90 μc of the radioisotope as the chloride in 0.9 per cent saline. Animals were killed in groups of three at intervals for 300 days following exposure. The radiocesium content of animals sacrificed at 300 days was too low to permit accurate analysis. Data are therefore included for the period 1 to 200 days only.

Drinking water containing 2×10^{-3} μc/ml of Cs^{137} is being fed *ad libitum* to 80 rats in the long-term chronic exposure experiment now in

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progress. Animals will be killed at intervals over a 500-day continuous exposure period.

Samples taken for analysis were digested in concentrated nitric acid and aliquots of the solution counted with an end-window Geiger tube. Appropriate corrections were made for self absorption, where necessary.

RESULTS AND DISCUSSION

The retention of Cs^{137} in several representative tissues is shown in Figure 1. The data have been normalized to a standard tissue and organ weight to avoid the dilution effect of growth. Retention curves for each tissue were plotted on semi-log paper and resolved into exponential components. Two components with biological half-lives of between 6 to 8 days and 16 to 18 days appeared to be present in all tissues. These two components account for about 85 per cent of the total cesium in the rat. The remaining 15 per cent is present in components of 2 to 4 day half-life which contain the major portion of Cs^{137} present in the visceral organs.

The retention curve for muscle and its resolution into components of 8 and 16 day half-life is shown in Figure 2. Also shown is a broken line representing the single exponential curve which best describes the loss of over 99 per cent of the isotope from muscle. The "average" biological half-life of 13 days and fraction of total cesium in muscle, of 0.57, determined by this curve, compare with the values presently employed by the NCRP and ICRP of 17 days for the half-life and 0.48 for the fraction in the critical organ (3,4).

Assuming muscle to be the critical organ, an MPC for water of 1.8×10^{-3} μc/ml results from calculations based either on the 13-day average half-life or on two components of 8 and 16-day half-life. Essentially the same value for the MPC is obtained if kidney is assumed to be the critical organ. The present ICRP value is 2×10^{-3} μc/ml which is rounded off from a calculated value of 1.7×10^{-3} (4).

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Results from the chronic exposure experiment now in progress show cesium concentrations to be increasing in all tissues after 100 days on the drinking water regimen. Muscle and kidney concentrations are highest, amounting to about ten and eight per cent of the average daily dose per gram of tissue.

ACKNOWLEDGEMENTS

The technical assistance of Joan O. Hess and Margaret B. Lawson is gratefully acknowledged.

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1086599

X IRRADIATION OF THE INTESTINE

M. F. Sullivan, P. L. Hackett and S. Marks

The LD_{50}^{30} of rats exposed to intestinal x irradiation, after surgical exteriorization of the gut, was determined as 1,550 r. This value following abdominal exposure was 1,620 r. The difference was not significant. Hematological analysis indicates that blood damage is more severe when the intact animal is irradiated. Pathologic damage is greatest from three to six days post-irradiation and is still evident 45 days after exposure to 1,500 r.

The gastrointestinal tract has been designated as the critical organ for the radio-toxicity of many of the radioisotopes that are poorly absorbed from the intestinal tract (1). In order to evaluate radiation damage to the digestive tract, a study was made of the effect that exposure of this organ has on processes that might be associated with intestinal injury.

METHODS

Three hundred eighty female Sprague-Dawley rats weighing between 200 and 240 g were used in these experiments. Animals anesthetized with Nembutal* were exposed to various doses of 250 kvp x-ray (HVL 1.15 mm Cu). Rat intestines which were surgically exteriorized were exposed to x radiation on a cardboard plaque, the remainder of the body being incased in a one-half inch thick cylindrical, half shell-type, lead shield. After the exposure, the G.I. tract was replaced, the fascia sutured, and skin closure made with skin clips.

Rats exposed to abdominal irradiation were placed on their side in a rectangular lead shield, one-half inch in thickness, with an aperture for exposure of the intestinal region. The region of the animal receiving direct irradiation extended from the greater trochanter, anteriorly. The

*pentobarbital sodium (Abbott)

vertebral column, kidneys, spleen and almost the entire liver were not directly exposed to radiation. Surgical and/or normal control animals were run in parallel with the irradiated animals.

At desired time intervals the animals were bled, via tail vein, for hematological analysis. Sacrifices were made at various periods after exposure for histological examination of the gastrointestinal tract. Prior to sacrifice, blood was withdrawn by cardiac puncture for culturing on both nutrient and blood agar. The intestine was sectioned at measured distances from the ileo-cecal junction, fixed in Bouin's solution, and stained with hematoxylon-eosin tissue stain.

RESULTS AND DISCUSSION

Results of the acute toxicity studies are graphed in Figure 1. The LD_{50} for acute abdominal x irradiation is approximately 1,620 r and after surgical exteriorization this value is 1,550 r, as determined by probit analysis (2). The difference between these values is not statistically significant. The majority of deaths occurred on the sixth post-irradiation day. Survival time has been used as a criterion of intestinal irradiation death (3, 4). Results of blood culture studies indicate that bacteria do not invade the blood stream during the acute intestinal phase of the radiation syndrome.

Hematological analysis was made on blood obtained from the tail vein of rats whose intestines were irradiated either in situ or surgically exteriorized. Control rats for animals whose intestines were irradiated after surgery were also surgically treated. Animals exposed intact are compared to normal controls. Thus all changes observed are due to the effects of radiation. Control data were subjected to a rectification technique to eliminate fluctuations arising from stimuli other than the experimental treatment. Leukocyte counts from animals exposed to a sublethal, median lethal or a lethal dose of x-ray are shown in Figure 2. These results show that there is a dosage dependent response at these levels of exposure. It can be seen in Figure 3 that the principal cell fraction responsible for

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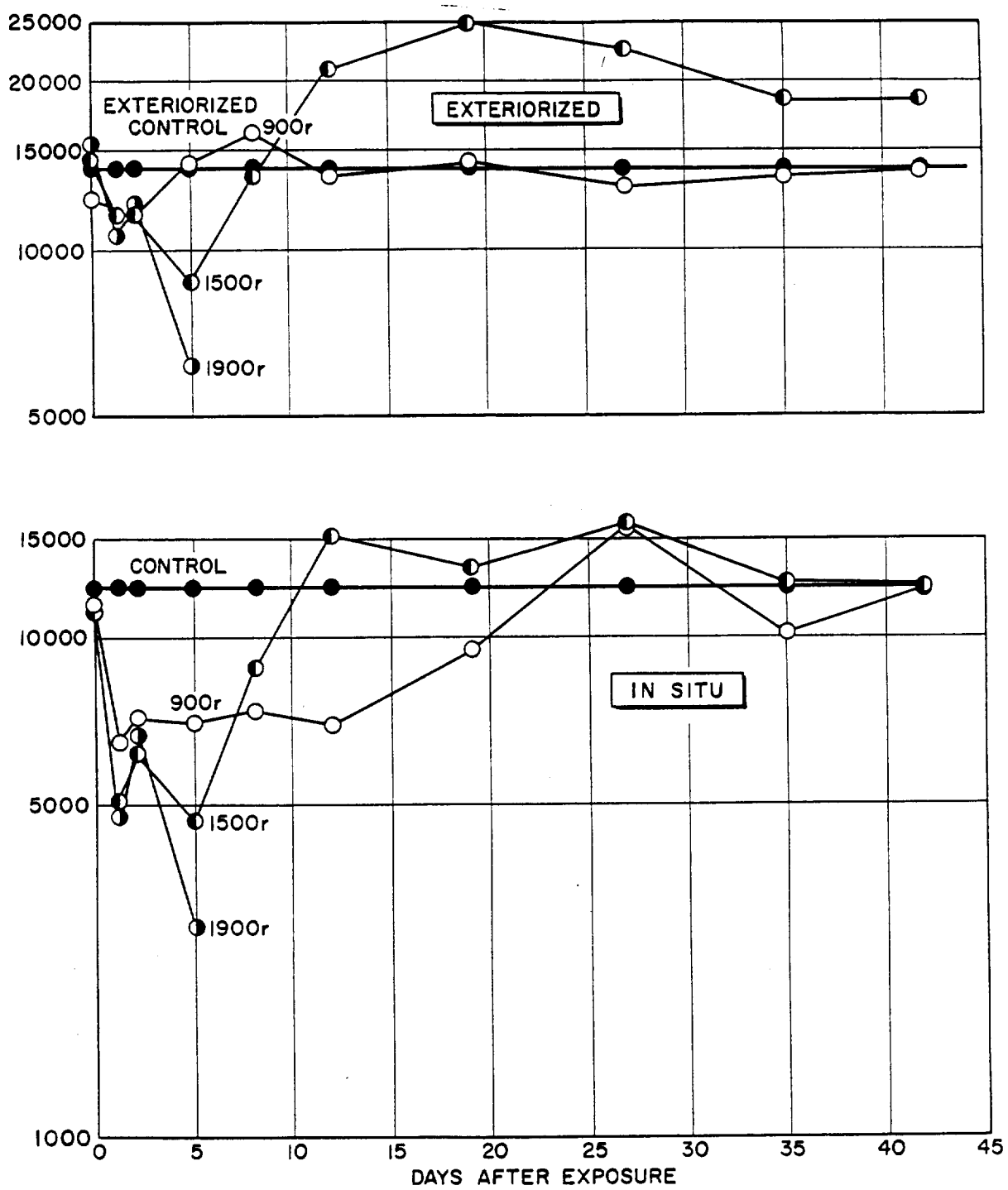

FIGURE 2

Effect of the Intestinal, Exteriorized or in situ, x irradiation on the Leukocytes of the Peripheral Blood of Rats.

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leukocyte depression is the lymphocytes. The sensitivity of lymphocytes to radiation is well established. However, it is apparent that in situ exposure caused a greater degree of lymphocytopenia with doses comparable on an LD_{50} basis. The increased leukocyte damage seen after in situ exposure may be due, in part, to the effect of "scatter" radiation on adjacent hematopoietic tissue. Results obtained from granulocyte counts are shown in Figure 4. Exposure of the surgically exteriorized intestine does not cause a decrease in granulocyte count in contrast to total body x-radiation (5). A marked granulocytosis occurs in the animals surviving 1,500 r on about the twelfth day after x-ray. This rise is more transient in the group that was exposed intact. Measurements of the hemoglobin content of the blood are shown in Figure 5. The increased values obtained after irradiation of the surgically exposed intestine indicate greater fluid loss and hemoconcentration. This effect is more marked when comparison is made with normal, rather than surgically-treated control animals.

Pathologic damage was similar in the exteriorized and in situ irradiated intestines. Damage was more severe and consistent in the ileum than in either the jejunum or colon. The epithelium of the crypts of Leiberkühn and the germinal centers of the lymphatic nodules were primarily affected. The sublethal dose (900 r) caused transient epithelial nuclear enlargement and mitotic inhibition. Six days after exposure the only residual effect was slight acute inflammation in the mucosa. Irradiation with either 1,500 r or 1,900 r caused changes in the intestine of similar character. The rate of development and the severity were more pronounced with the higher dose. One day after irradiation karyorrhexis was present in the germinal centers of the lymphatic nodules. Mild nuclear enlargement and pallor were evident in the epithelium of the crypts, and mitotic inhibition was present. Two days following exposure there were mitotic inhibition, early acute inflammation and a variation in cellular morphology which became severe on the third day. At this time bizarre shaped cells were in evidence. Debris from lymphatic damage had been removed by this time and germinal centers

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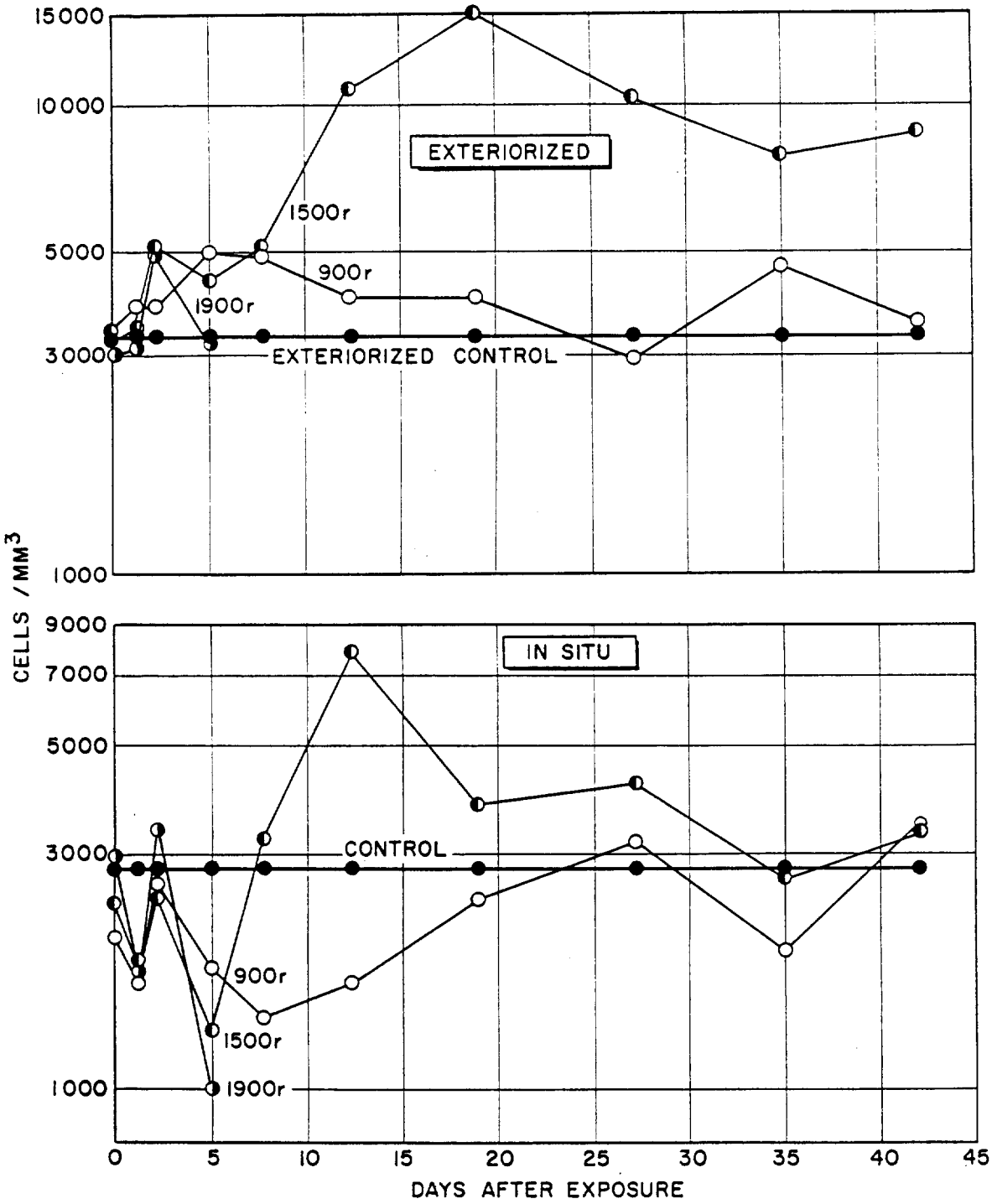

FIGURE 4

Effect of Intestinal x irradiation on the Granulocytes of the Peripheral Blood

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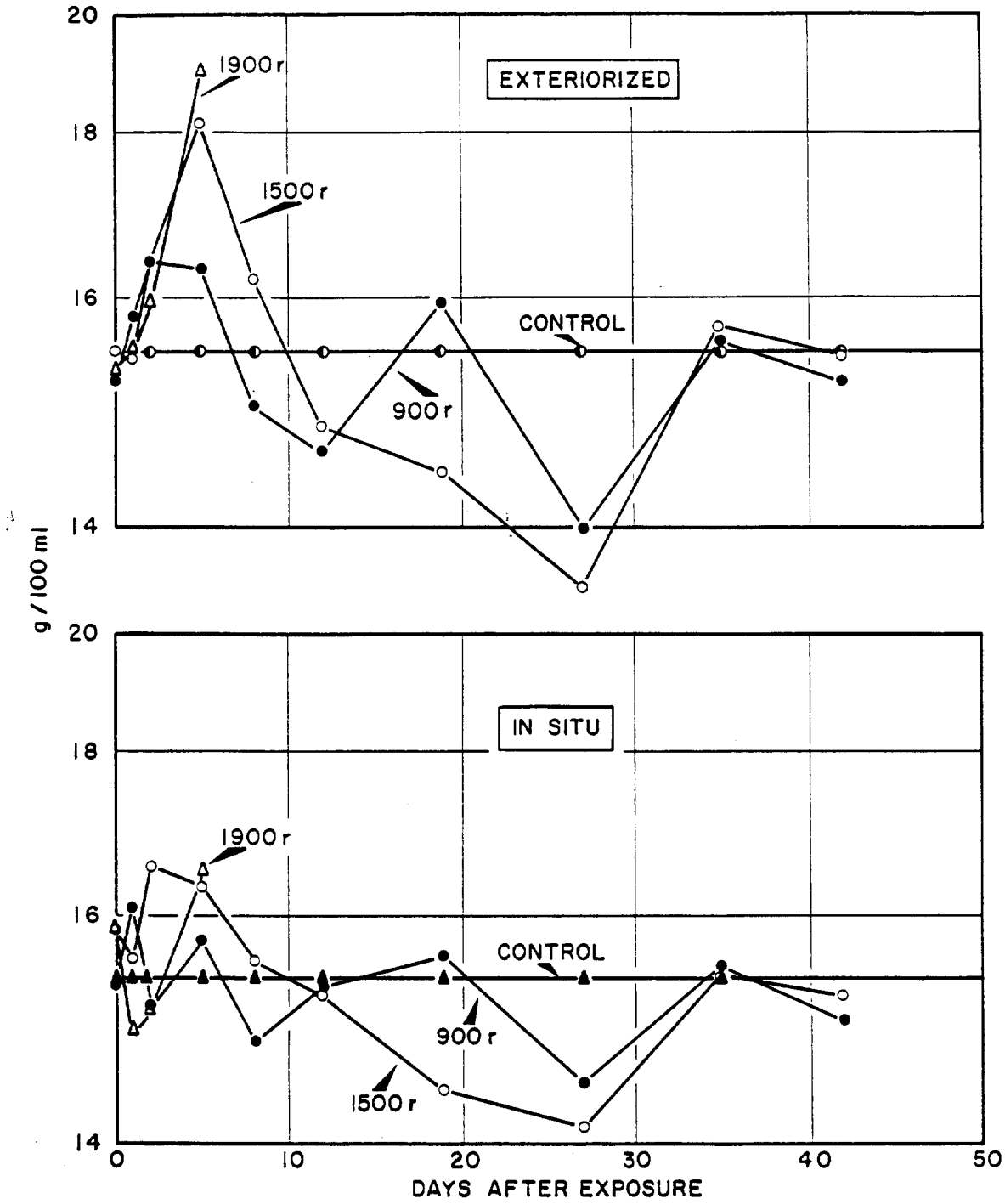

FIGURE 5

Effect of Exposure of the Intestine of Rats to x ray on the Hemoglobin Content of the Peripheral Blood

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had disappeared. By the sixth day the crypts were dilated and lined with flattened amitotic epithelium. Ulceration of the mucosa was present, especially in the 1,900 r group. Crypts having a relatively normal appearance, which were assumed to represent a regenerative process, were initially observed in small numbers on the sixth day. Subsequent time periods up to 45 days in the 1,500 r groups showed rapid healing by means of the replacement of damage by regenerated crypts. Isolated cystic crypts and mucosal ulcerations constituted a late residium of damage in animals exposed to 1,500 r.

The hematological changes observed following x irradiation of the intestine, in situ or after surgical exteriorization, are similar to those shown after whole-body exposure. However, in contrast with total-body irradiation, the granulocytes are only slightly depressed. This difference may explain the absence of bacteremia commonly associated with whole-body x irradiation. Although abdominal irradiation appears to cause more severe blood damage, a valid comparison of either hematological or histological changes cannot be made without accurate knowledge of the absorbed dose. Damage to the blood constituents shown in this study and elsewhere in this report (6, 7) may appreciably influence the resistance of the animals to the toxic effects of intestinal irradiation, but the histological damage observed is, no doubt, the primary cause of death.

ACKNOWLEDGEMENTS

The technical assistance of Alma Crosby, and members of the Biological Analyses Operation is gratefully acknowledged.

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TABLE 1

Acute Toxicity of Radio-Yttrium to Rats after Oral Administration

ose ıc/kg)	Mortality (30 days)	Mortality %	Time of death (Day after adm.)
10	0/8	0	
15	5/13	38.5	10.
20	5/6	83.3	8.0
25	3/3	100	8.0
30	4/4	100	9.0
40	2/2	100	5.5

ɔm these results the LD_{50}^{30} was estimated at 17 mc/kg. Survival time been accepted as a criterion of intestinal radiation injury (2). Mortality dies have shown that the average time of death for rats is about six days ɜr exposure of the G. I. tract to x radiation (3). Although more of the ɜstine is exposed to radiation by an internal emitter, death did not occur m Y^{91} until an average of nine days after ingestion. However, the radia- ı dose to the intestine from this internal emitter was delivered until it ; excreted. As the Y^{91} dose increased the survival time was decreased. liochemical analysis of the bone taken from rats that had received lethal es of Y^{91} showed that only 0.03 per cent of the administered dose was orbed. This amount is in close agreement with the accepted value (4), indicates that the amount absorbed is not of sufficient magnitude to be tely toxic. Therefore, death is due to acute beta irradiation of the G. I. ɜt.

Hematological analysis of the peripheral blood of rats was made ɔr to, and at various time intervals after isotope administration. Results eukocyte counts made on blood obtained from Y^{91} treated rats are shown 'igure 1. Data from control rats were subjected to a rectification technique liminate fluctuations not due to experimental treatment. A dose of

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10 mc/kg appears to depress the white cell count about one-half as much as either 15 or 25 mc/kg. However, recovery was slower from the smaller dose. This deviation from the dose dependent effect expected is not due to the mononuclear fraction as shown in Figure 2. After 15 or 25 mc of Y^{91}, lymphocyte numbers are depressed to the greatest extent at three days after administration. It appears that recovery of the lymphatic tissue is well-advanced when death occurs. Granulocyte counts (Figure 3) increase markedly within the first week after treatment. The rapid increase in leukocyte count is due in part to hemoconcentration as shown in Figure 4. Hemoglobin values are elevated on the fifth day but are normal ten days after ingestion. In Figure 5 it can be seen that erythrocyte counts are elevated during the first six days but decrease markedly by the eighth day. Red cell counts returned to normal two weeks after Y^{91} administration. Reticulocyte counts shown in Figure 6 are decreased three days after yttrium ingestion and remain depressed until the twelfth day.

Preliminary mortality data obtained from the administration of Pu^{239} per os are shown in Table 2. These data suggest that the LD_{50} is about 90 μc/kg. When compared on a mc/kg basis Pu^{239} is about one-fifth as toxic as Y^{91}. On an energy release basis plutonium is only one-fiftieth as toxic. Death occurs on the first day following plutonium administration rather than on the ninth day as in the case of radio-yttrium.

TABLE 2

Acute Toxicity of Plutonium to Rats After Oral Administration

Dose (mc/kg)	Mortality (30 days)	Mortality (%)	Time of death (Day after adm.)
55.5	0/6	0	
88.3	1/4	25	1
92.6	2/4	50	1
100	2/2	100	1

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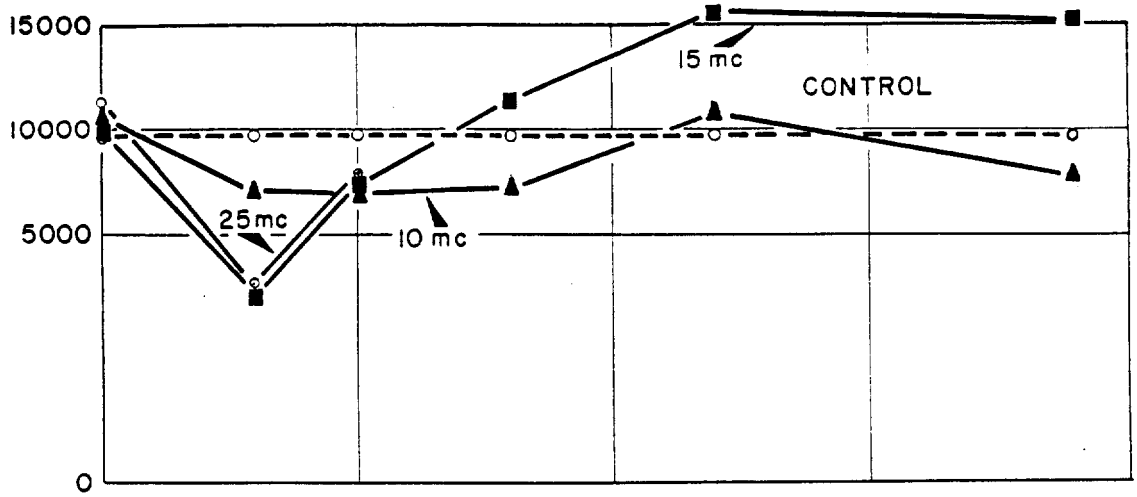

FIGURE 1
Effect of Orally Administered Y^{91} on the Leukocytes of the Peripheral Blood of Rats

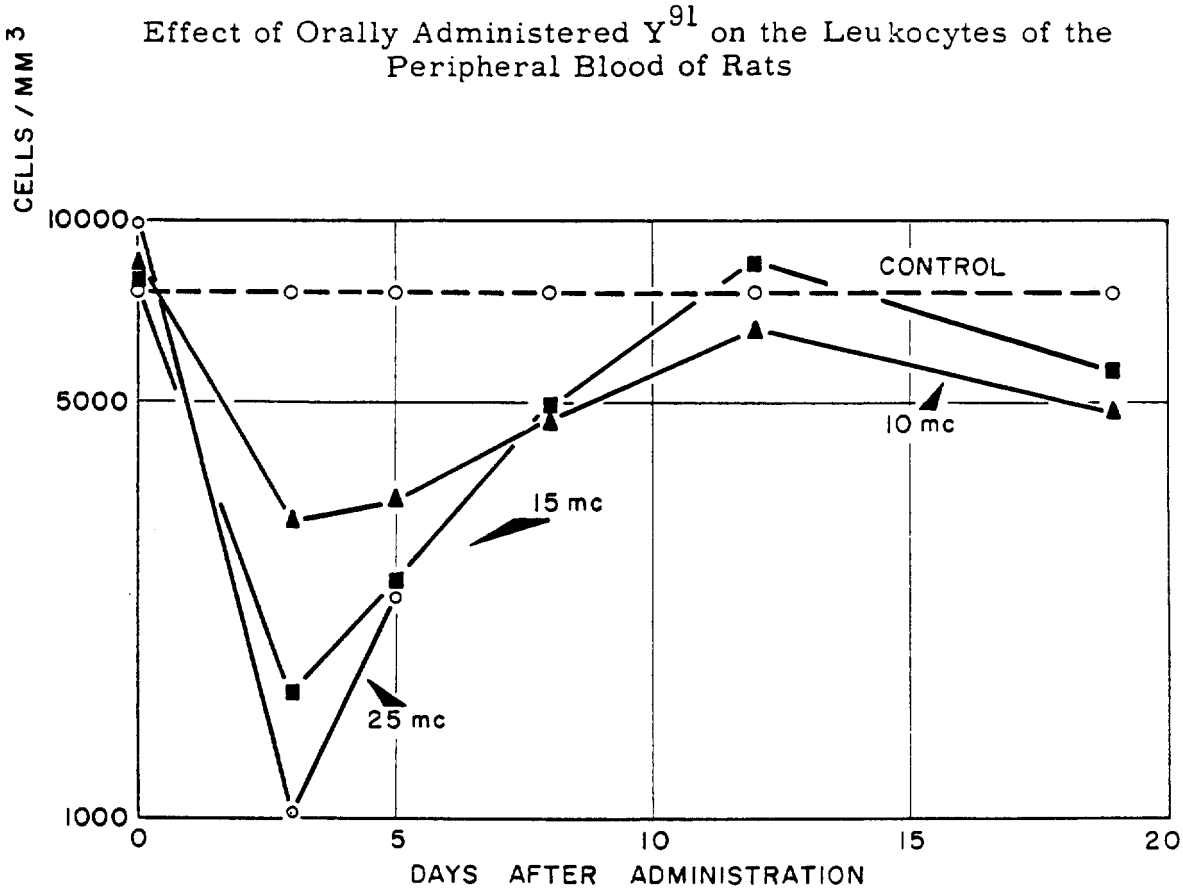

FIGURE 2
Effect of Orally Administered Y^{91} on the Lymphocytes of the Peripheral Blood of Rats

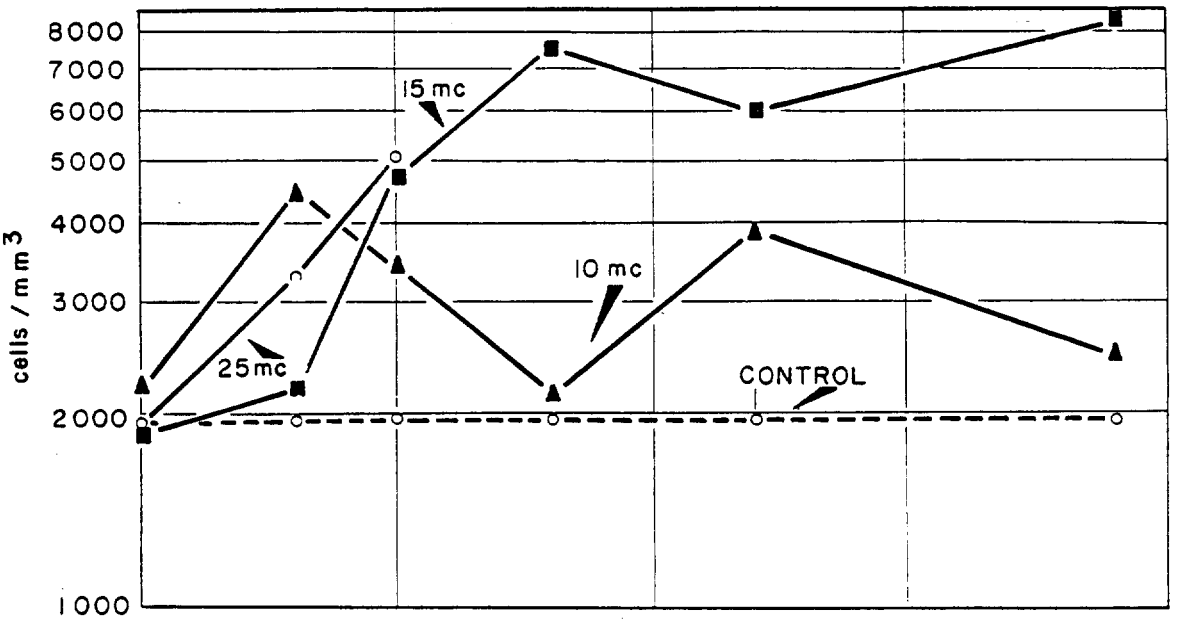

FIGURE 3

Effect of Orally Administered Y^{91} on the Granulocytes of the Peripheral Blood of Rats.

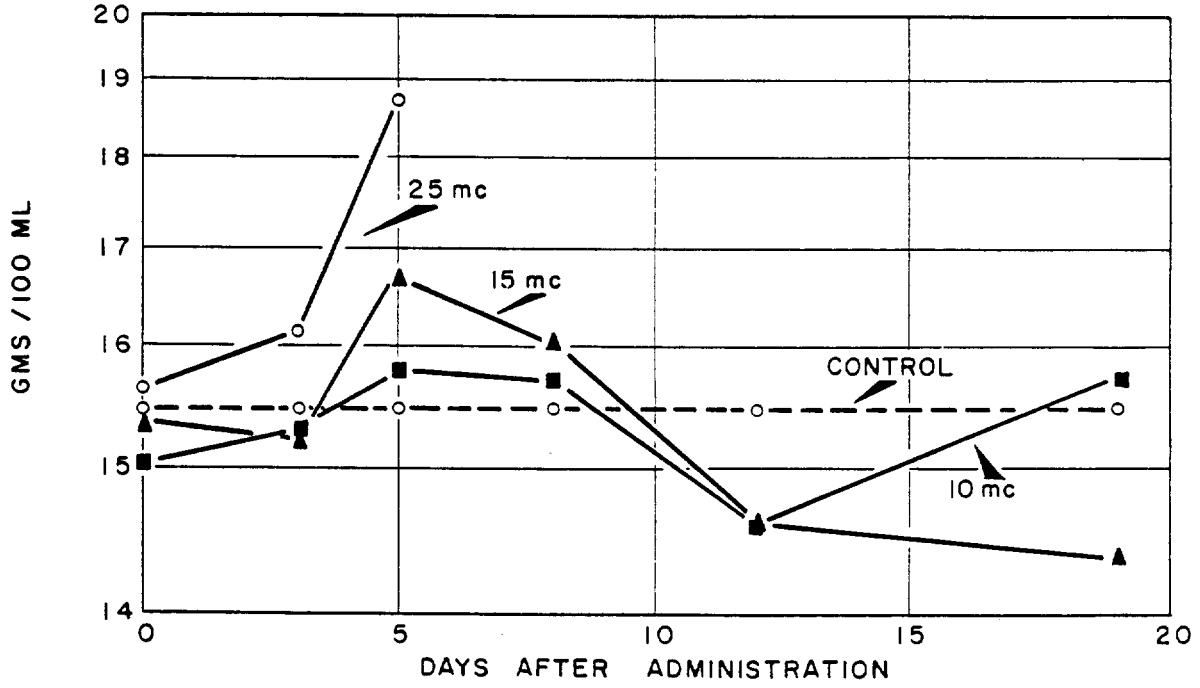

FIGURE 4

Effect of Orally Administered Y^{91} on Hemoglobin Values of the Peripheral Blood of Rats.

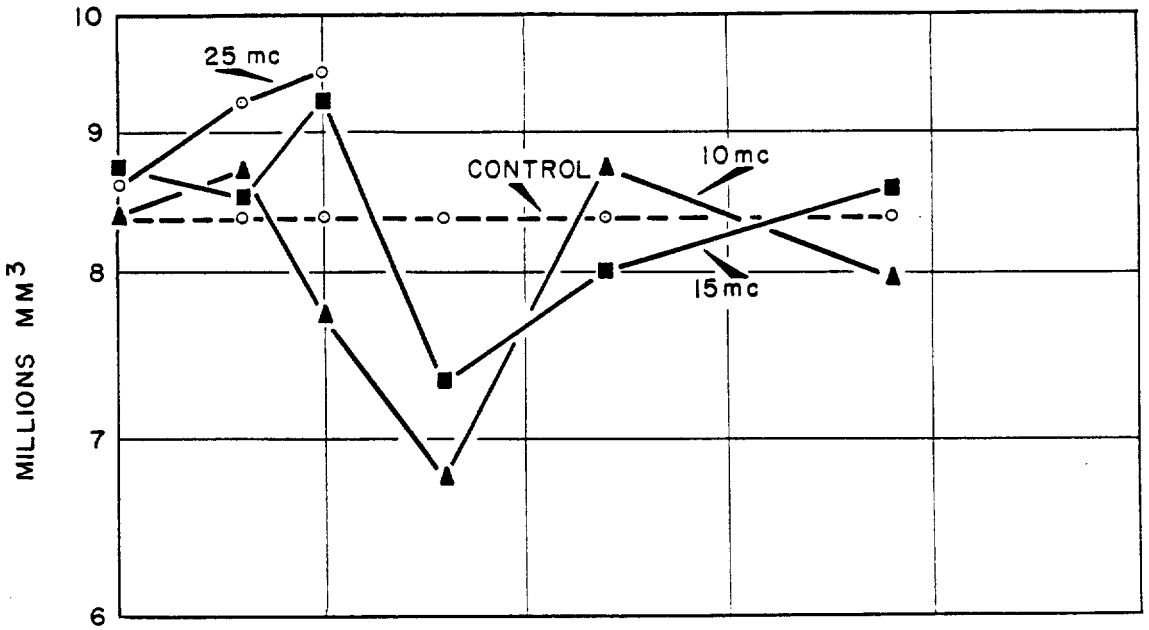

FIGURE 5

Effect of Orally Administered Y^{91} on the Erythrocytes of the Peripheral Blood of Rats.

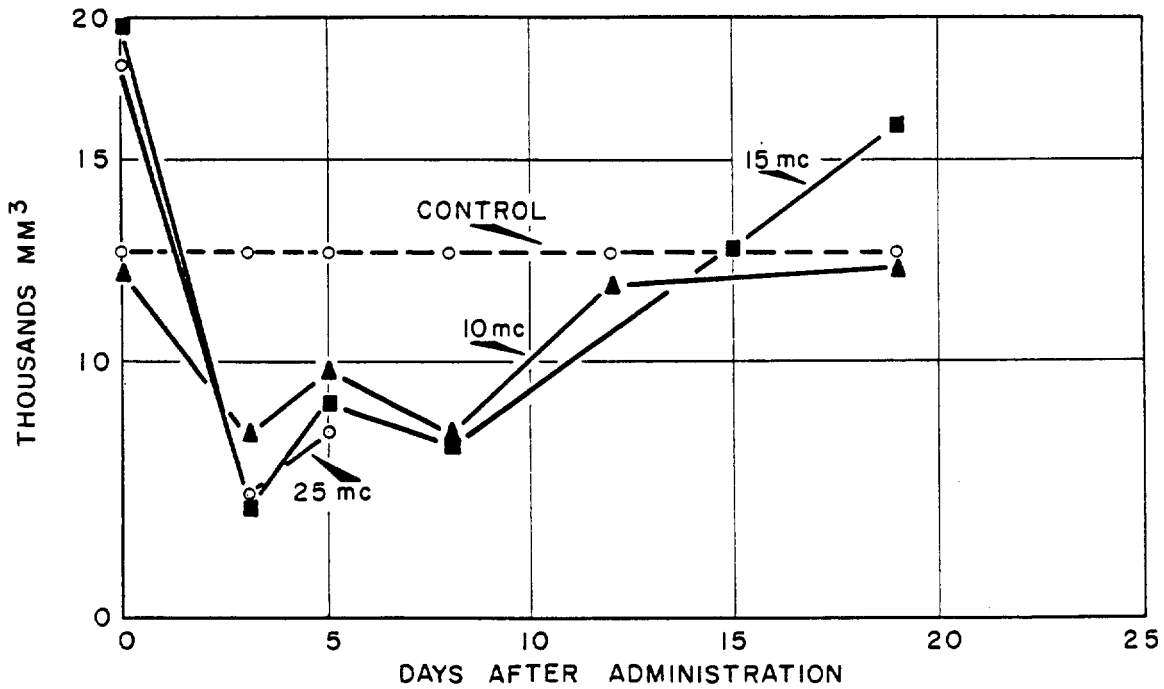

FIGURE 6

Effect of Orally Administered Y^{91} on the Reticulocytes of the Peripheral Blood of Rats.

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EFFECT OF INTESTINAL TRACT IRRADIATION ON SERUM PROTEINS

R. F. Palmer and M. F. Sullivan

Electrophoretic fractionation of the sera of rats following exposure of their intestinal tracts to x radiation showed marked effects on the serum protein pattern. Oral administration of Y^{91} showed similar, but somewhat delayed effects. Decrease in serum albumin was used to correlate dosage and its effect on serum proteins.

Several authors have reported that the protein composition of rat serum is altered after whole-body x irradiation (1, 2). When the intestinal tract irradiation studies described elsewhere in this report were initiated (3), it was felt that a study of the effects of the various types and methods of intestinal tract irradiation on the serum proteins might shed some light on the mechanism of radiation damage to the intestinal tract.

This paper briefly describes the results obtained by paper electrophoretic fractionation of the proteins in the sera of animals at various times following x-irradiation of either the whole body, or the intestinal tract - both in situ and exteriorized. Results are also included from animals that received beta irradiation of the G. I. tract from the administration of Y^{91} by stomach tube.

METHODS

Eighty-three female Sprague-Dawley rats weighing between 200 and 240 g were used in these experiments. The irradiation procedures are described elsewhere in this report (3).

The electrophoretic characterization was done by the Durrum method (4), using Veronal buffer of pH 8.6 and 0.1 ionic strength. The protein composition of the serum was determined by scanning the dyed filter paper strips in the Spinco Analytrol Photometer-Computer.

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RESULTS AND DISCUSSION

In all groups of irradiated animals the per cent albumin in the sera showed the largest and most consistent changes following irradiation. This consistency was probably due to the better precision of interpreting the "Analytrol" trace for per cent albumin than for the other serum protein fractions. To present the dose dependency of the composition of the serum proteins as clearly as possible, the per cent albumin in the irradiated animal sera was divided by the average of the per cent albumin in the control animal sera fractionated in the same electrophoresis "run". This quotient, which is expressed as $\%A/\%A_c$, tends to minimize the variations in the electrophoresis procedures.

Figure 1 shows the variation with time and dose of the average $\%A/\%A_c$ values of the sera from rats whose intestinal tracts were irradiated in situ. Each point is the average of results from 3 to 9 animals. The reason for the apparent discrepancy in results from one- and two-day animals that received 1,900 r is not known. It is apparent from Figure 1 that the variation of $\%A/\%A_c$ with time is dose dependent.

The maximum depression in albumin level was observed 6 days after x irradiation. The time of maximum depression and the pattern of subsequent recovery is similar to the effect of intestinal irradiation on other blood elements (3). The amount of histological damage and time of maximum mortality caused by acute doses of radiation to the intestine suggest a relationship among these effects.

Although the decreased albumin level was caused by exposure of the abdominal region, the possibility remained that the liver may have absorbed sufficient "scatter radiation" to cause an inhibition of serum albumin synthesis at this site. Therefore, the serum protein pattern was determined in animals that were totally shielded except for an exteriorized portion of the intestinal tract which was irradiated (3). Results of these studies are shown in Figure 2.

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Each point is the average of results from 4 to 7 serially bled animals. It is apparent from these results that the decrease in serum albumin is approximately the same, whether the gut is irradiated exteriorized or in situ. This effect must therefore be due either to the direct action of radiation on the intestine, or to an indirect action by some material produced in the gut during irradiation.

Figure 2 shows the effect on serum albumin of internal beta irradiation of the G. I. tract. The 3 mc dose of Y^{91} administered is of comparable acute lethality to 1,500 r of x ray. Since yttrium is absorbed from the G. I. tract to a negligible extent, its effect must be attributed to irradiation of the intestines and closely adjacent tissues. Albumin is decreased following oral administration of Y^{91}, but the onset of this effect is delayed, and the magnitude decreased, as compared with x irradiation effects. This is probably related to the longer time required for the intestine to absorb a beta-radiation dose comparable to the x-ray dose. Exposure of rats to whole-body x radiation of comparable acute lethality (600 r) caused a much smaller effect on serum albumin than did the 1,500 r partial body dose.

The above findings show that irradiation of the intestinal tract affects the albumin content of rat serum in the same manner as does whole-body irradiation. As this effect, which has been attributed to irradiation of the liver during whole exposure, results from irradiation of only the intestinal tract, an indirect action by some material produced in the gut during irradiation is suggested.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the able technical assistance of Alma L. Crosby and John T. Homer.

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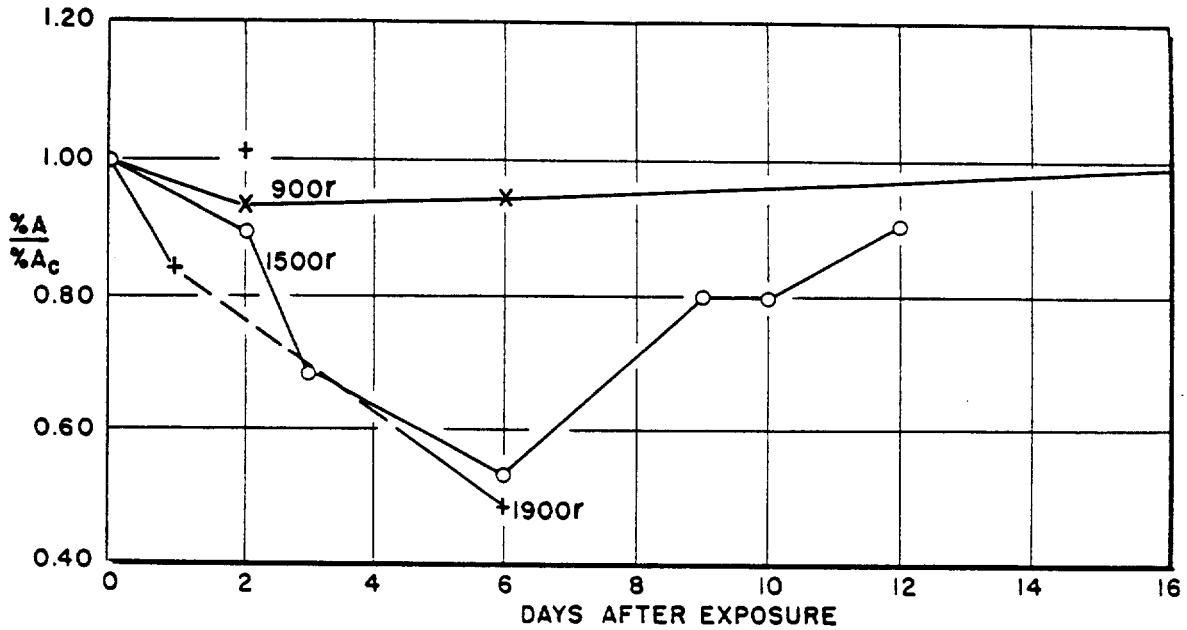

FIGURE 1

Effect of in situ Intestinal x irradiation on the $\%A/\%A_c$ Value in Rat Serum

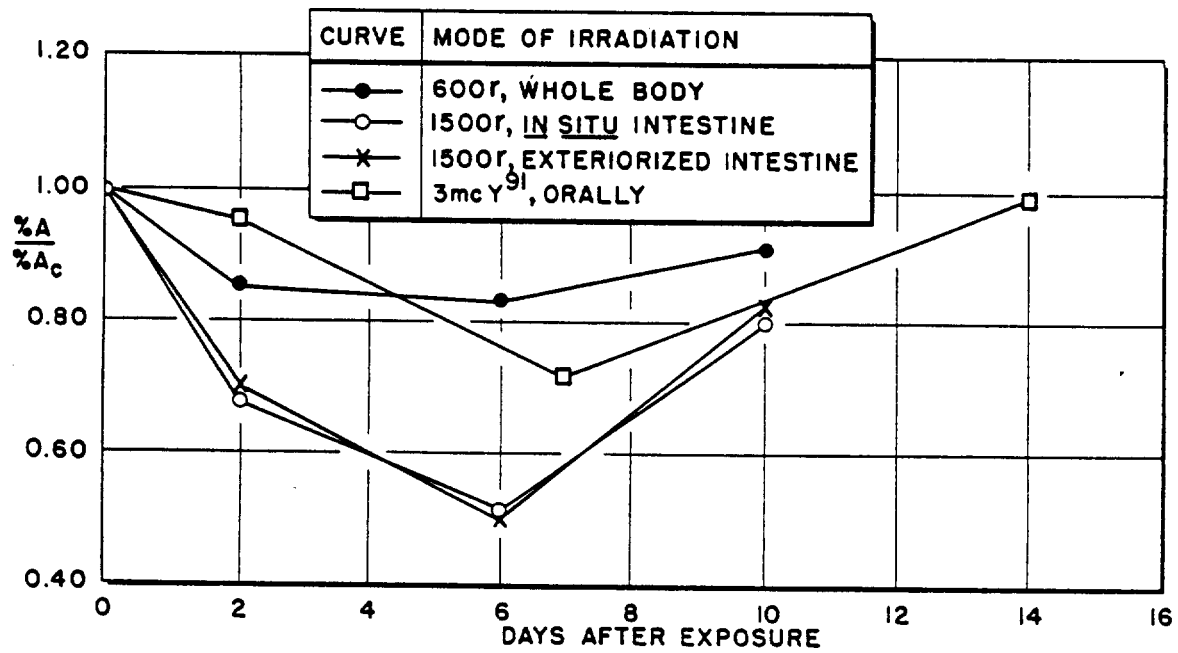

FIGURE 2

Effects of Various Modes of Irradiation on the $\%A/\%A_c$ Value in Rat Serum

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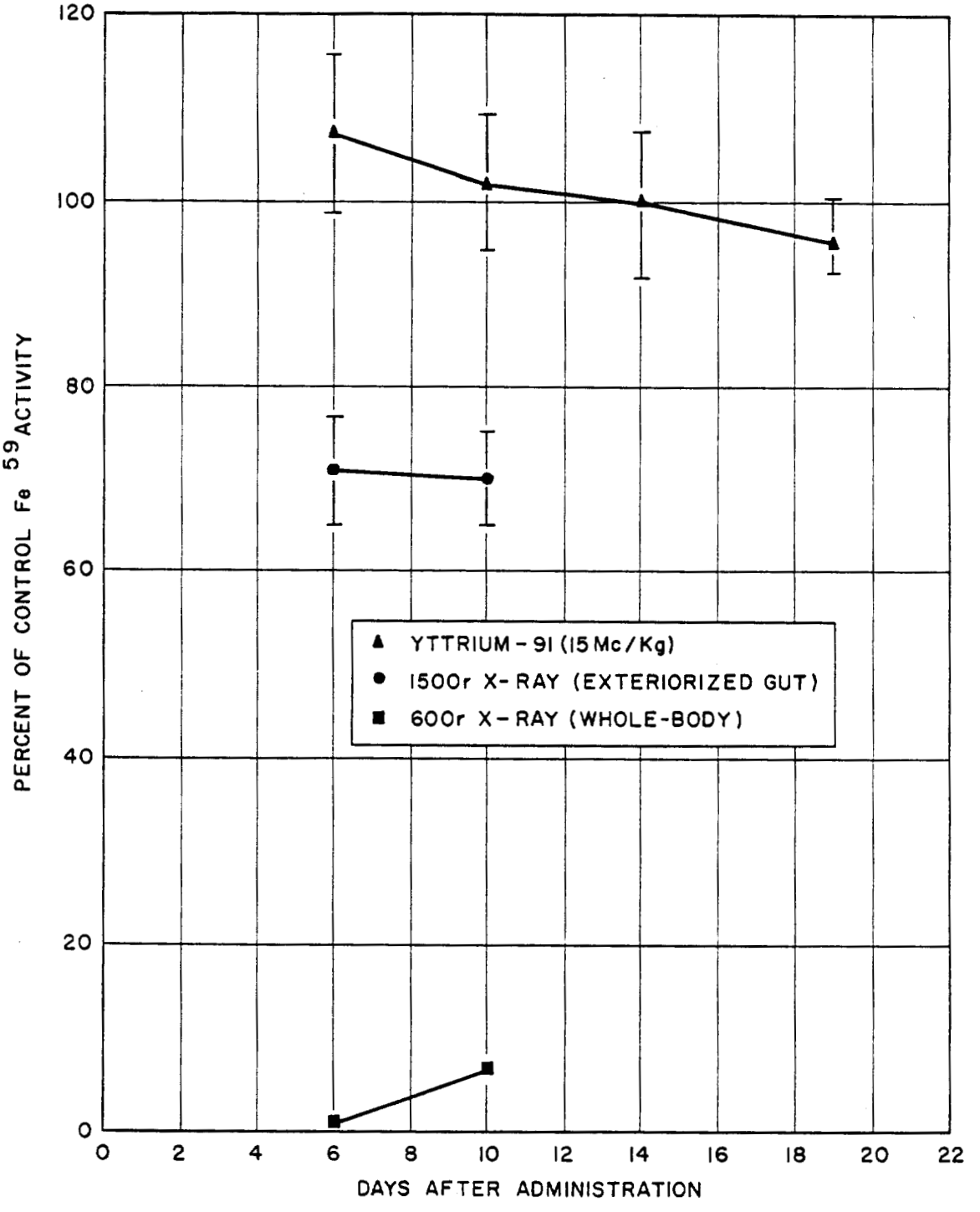

FIGURE 1

Uptake of Fe^{59} in the Circulating Erythrocytes of Irradiated Rats after Intravenous Injection

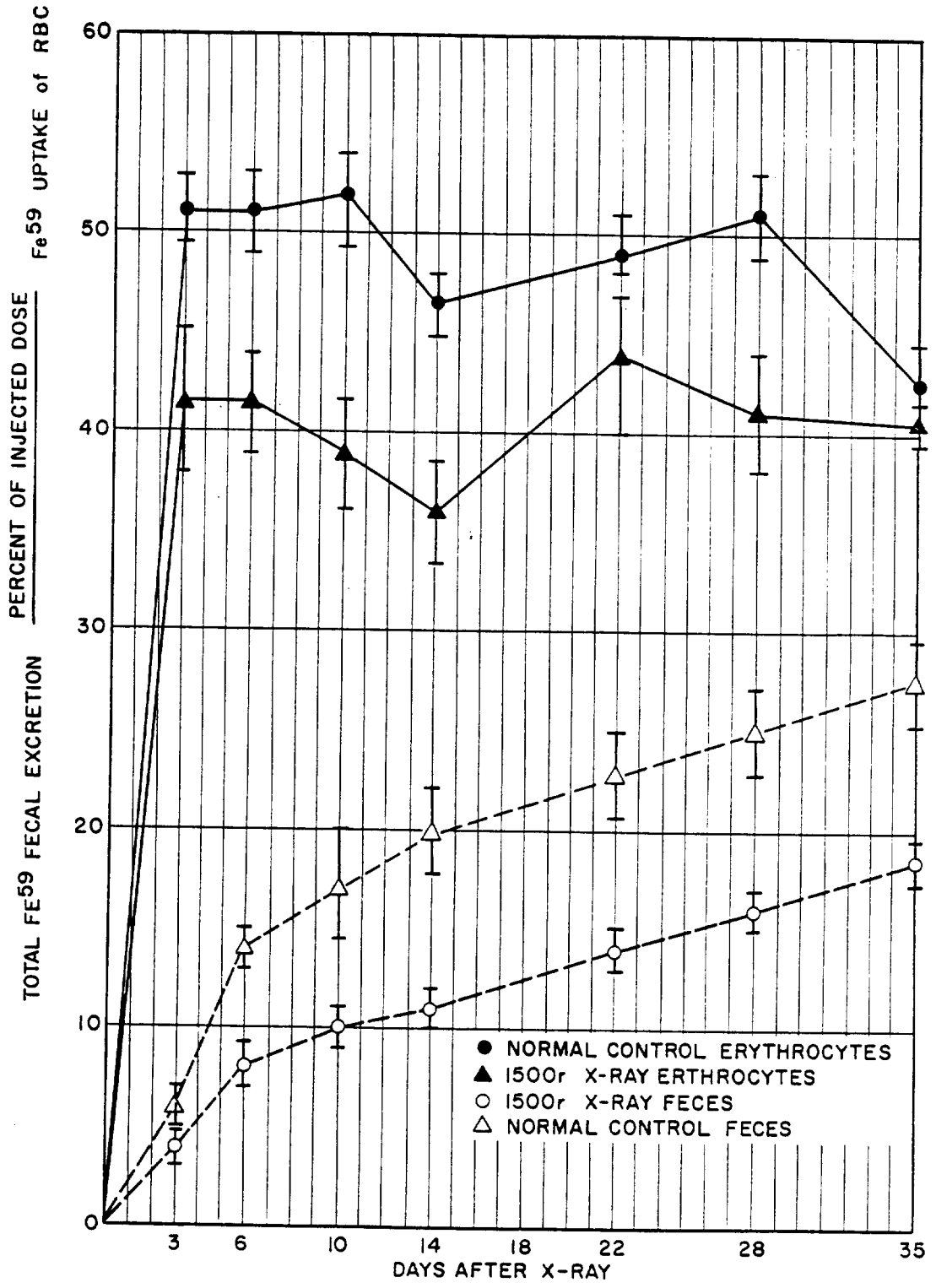

FIGURE 2

Rat Erythrocyte Uptake and Cumulative Fecal Excretion of Fe^{59} after 1500 r of Abdominal X-Ray Exposure.

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1086619

EFFECTS OF ISOTOPIC DILUTION ON Ca^{45} AND Sr^{89} DEPOSITION

B. Kawin

Intraperitoneal and intragastric administration of stable calcium or strontium in amounts up to 3 mg/g body weight did not decrease the fraction of simultaneously administered Ca^{45} or Sr^{89} deposited in rat femur, liver, kidney, or spleen. Under certain conditions the radioisotope deposition was increased when administered with stable calcium or strontium.

In continuation of a previous investigation (1) of the influence of non-radioactive carrier upon the distribution of radiocalcium and radiostrontium in rats, additional animals have been utilized and the study expanded to include both oral and intraperitoneal administration.

METHODS

Five hundred sixteen young adult female Sprague-Dawley rats were intraperitoneally or intragastrically injected with 10 μc of either Ca^{45} or Sr^{89} in solutions containing various concentrations of stable calcium or stable strontium. Twenty-four hours after injection the animals were killed, and the femur, liver, kidneys, and spleen were assayed for Ca^{45} or Sr^{89}.

RESULTS AND DISCUSSION

The added metal ions did not reduce, and in some instances, increased the concentrations of the radioisotopes in the tissues. The significant effects noted are shown for intraperitoneal administration in Table 1, and for intragastric administration in Table 2. These tables indicate three significant levels at which increased uptake of Ca^{45} or Sr^{89} was found to be related to concentration of carrier. The information in Tables 1 and 2 does not

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indicate the magnitude of the effects, or the levels of added calcium or strontium required to elicit the effects.

Intraperitoneal Administration

The over-all trend exhibited by Table 1 indicates a dependence upon carrier in soft tissues if radioactive metal is intraperitoneally injected. No substantial significant effects were observed on femur concentration of either Ca^{45} or Sr^{89}. In agreement with the previous study (1) the Ca^{45} concentration in liver increased with administration of either stable calcium or strontium. The Ca^{45} concentration of kidney increased about ten-fold, and about two-fold in the case of liver. Significant effects of added stable isotope on Sr^{89} were much less pronounced. Threshold toxic levels were approximately 90 μg/g body weight for calcium, and approximately 300 μg/g body weight for strontium.

Intragastric Administration

After oral administration the soft tissues accumulated proportionately much less of the radioisotopes than the femur, and the concentrations of the radioisotopes in all tissues were quantitatively less than after intraperitoneal injection. As shown in Table 2, stable calcium and strontium administered orally with Ca^{45} increased its deposition in femur. The increase in femur deposition associated with stable strontium was almost four times that observed with calcium carrier. In contrast, stable calcium, but not stable strontium, increased deposition of Sr^{89} in femur. The proportionately large amounts of Ca^{45} and Sr^{89} found in femur after absorption from the gastrointestinal tract indicated preferential retention of the radioisotopes by the skeleton as compared to the soft tissues.

The results of this study indicate no reduction of deposition of radiocalcium or radiostrontium in tissues as a consequence of dilution with their non-radioactive isotopes. This would seem to indicate that the capacity of the tissues for incorporation of calcium and strontium is not exceeded

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TABLE 1

Degree of Certainty of Increase in Concentration in Rat Tissues of Ca^{45} and Sr^{89} Following Intraperitoneal Administration with Stable Calcium and Strontium[a]

Radioisotope[b]	Ca^{45}		Sr^{89}	
Stable Isotope Added [c]	Ca	Sr	Ca	Sr
Femur[d]	*	0	0	0
Liver	***	**	0	0
Kidney	**	***	*	*
Spleen	0	***	*	0

[a]The values on which these tables are based were obtained at 24 hours post-injection.

[b]The Ca^{45} solutions without added salt contained 14 to 130 μg Ca per Ca^{45}. The Sr^{89} solutions were carrier-free.

[c]Stable calcium was added as $CaCl_2 . 2H_2O$ in quantities ranging from 0 to 3050 μg Ca/g rat. Stable strontium was added as $SrCl_2 . 6H_2O$ in quantities ranging from 0 to 1260 μg Sr/g rat.

[d]The asterisks in each column indicate the degree of certainty that an increase in uptake of the radioisotope was obtained by addition of stable metal. "O" indicates no significant change in tissue concentration with addition of stable metal. "*" indicates 95% certainty, "**" indicates 99% certainty, and "***" indicates 99.9% certainty in radioisotope concentration.

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TABLE 2

Degree of Certainty of Increase in Concentration in Rat Tissues of Ca^{45} and Sr^{89} Following Intragastric Administration with Stable Calcium and Strontium[a]

Radioisotope[b]	Ca^{45}		Sr^{89}	
Stable Isotope Added[c]	Ca	Sr	Ca	Sr
Femur[d]	**	***	***	0
Liver	*	*	0	*
Kidney	*	0	0	**
Spleen	0	*	0	0

[a]The values on which these tables are based were obtained at 24 hours' post-injection.

[b]The Ca^{45} solutions without added salt contained 14 to 130 μg Ca per μc Ca^{45}. The Sr^{89} solutions were carrier-free.

[c]Stable calcium was added as $CaCl_2 . 2H_2O$ in quantities ranging from 0 to 3050 μg Ca/g rat. Stable strontium was added as $SrCl_2 . 6H_2O$ in quantities ranging from 0 to 1260 μg Sr/g rat.

[d]The asterisks in each column indicate the degree of certainty that an increase in uptake of the radioisotope was obtained by addition of stable metal. "0" indicates no significant change in tissue concentration with addition of stable metal. "*" indicates 95% certainty, "**" indicates 99% certainty, and "***" indicates 99.9% certainty in radioisotope concentration.

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in the range of physiologically tolerated concentrations (2). Isotopic dilution thus does not appear to be an effective means of minimizing the deposition of these radioelements in either skeletal or soft tissues.

ACKNOWLEDGEMENTS

The able technical assistance of D. S. DeChant and other members of the Metabolism Operation is gratefully acknowledged.

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1086624

TURNOVER OF INORGANIC VERSUS PLANT INCORPORATED SR^{90} TO SHEEP MILK

V. G. Horstman, A. A. Selders*, W. L. Nicholson,**
F. P. Hungate and L. K. Bustad

Amounts of Sr^{90} secreted in the milk of sheep were the same whether the strontium was orally administered as the nitrate or administered in plant material that had been grown on contaminated soil. When Sr^{90} as the nitrate was fed daily for fourteen days, apparent equilibrium was reached by the fifth day, and at that time about four per cent of the strontium ingested appeared in the milk.

Although extensive experiments have been conducted on the metabolism of radiostrontium administered in the inorganic form, no studies have been described comparing these metabolic data with those obtained from the administration of radiostrontium incorporated in plant material. In order to provide a basis for the extrapolation of data from studies on inorganic strontium to actual field contamination events, a study was performed extending observations previously made by Cline (1), comparing the concentrations of radiostrontium in milk of ewes fed either the Sr^{90} nitrate or Sr^{90} incorporated in barley hay. The material referred to as plant incorporated Sr^{90} was mature barley grown on land contaminated with this radioactive isotope (2, 3).

METHODS

Six ewes with single lambs were divided into three groups (Table 1). For the first phase, one group received 1.6 μc of Sr^{90} incorporated in barley straw and grain once each day, for three days. Another group received 1.3 μc of Sr^{90} nitrate in a drilled feed pellet once each day for

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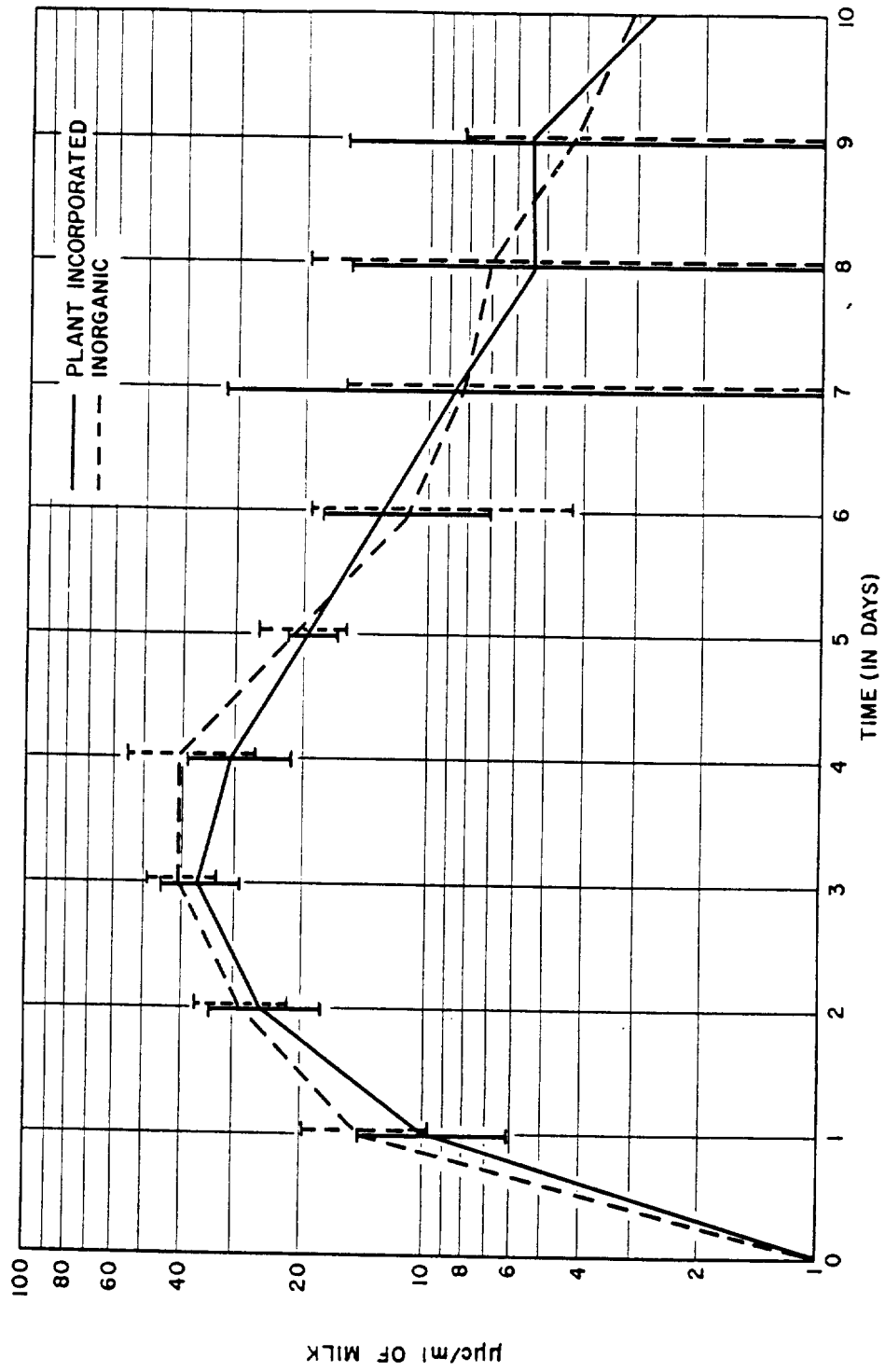

FIGURE 1

Sr^{90} in the Milk Following Feeding of Inorganic and Plant Incorporated Sr^{90}

The vertical lines show the 95 per cent confidence intervals for the mean concentration of Sr^{90} in the milk (based upon the range).
The concentration values for inorganic Sr^{90} were multiplied by the conversion factor of $\frac{1.6}{1.3}$ in order to make the two dose levels comparable.

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1086627

TOXICITY OF I^{131} IN SHEEP

XXII. EFFECTS FOLLOWING PROLONGED ADMINISTRATION

L. K. Bustad, V. G. Horstman, L. A. George, Jr.*, S. Marks**,
P. L. Hackett, W. J. Clarke, Jr., L. J. Seigneur, and H. A. Kornberg

This paper reports the progress on a chronic experiment designed to determine the minimum quantity of I^{131} that may be damaging when administered daily to sheep during their effective life. Reduction in thyroid avidity for I^{131} was noted in groups fed 0.5, 1.5, and 5 μc/day. The group fed 5 μc/day for over five years also showed thyroid adenomas and a reduction in serum protein-bound iodine.

Papers I, V, XIV and XIX in this series summarized the general observations made during 1950-1955 on a chronic experiment defining the biological effects of I^{131} in sheep following prolonged administration (1-5). This paper reports the general observations made during 1956 on the experimental animals fed 0.15, 0.5, 1.5 and 5 μc/day, and their offspring.

METHODS

The number of animals in the groups under investigation in January, 1956 was as follows:

TABLE 1

Number of Animals in Groups, 69 Months After Start of Experiment

	μc I^{131} fed/day				
	5	1.5	0.5	0.15	Control
No. of 1950 offspring	4			6	5
No. of 1951-1952 offspring	4	4	4	8	5
No. of 1954 offspring	3	6	3		
No. of 1955 offspring		4	4	4	5

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In addition to the animals which are subjects of the main chronic study a group of adult ewes was fed 5 μc of I^{131}/day until sacrificed at graded intervals to determine when damage is first exhibited in the thyroid. One animal was sacrificed after one year of feeding and another after one and a half years of feeding. Necropsies were also performed on all 1950 offspring as they approached six years of age, and on two animals from the 1951-1952 offspring fed 0.15 μc/day.

The following animals and groups were under study in December, 1956:

TABLE 2

Number of Animals in Groups, 81 Months After Start of Experiment

	μc I^{131} fed/day				
	5	1.5	0.5	0.15	Control
No. of 1951-1952 offspring	4	4	4	6	5
No. of 1954 offspring	2	6	2		
No. of 1955 offspring		4	4	4	5

Weekly external monitoring of all sheep thyroids was continued. Periodic blood samples were collected from all ewes. Hematological procedures included leukocyte count, differential count, hemoglobin, and packed cell-volume determination. Blood chemistry procedures included creatinine, protein-bound iodine, inorganic phosphorus, and cholesterol determinations. Tissue samples were taken at necropsy for routine radiochemical analysis and histologic study.

RESULTS AND DISCUSSION

External Thyroid Monitoring

The thyroid function, expressed as the mean ratio of the quantity of I^{131} in the thyroid to the quantity of I^{131} fed daily, Q/D, is shown in Figure 1 and Figure 2 for the 1951-1952 offspring, and in Figure 3 for the

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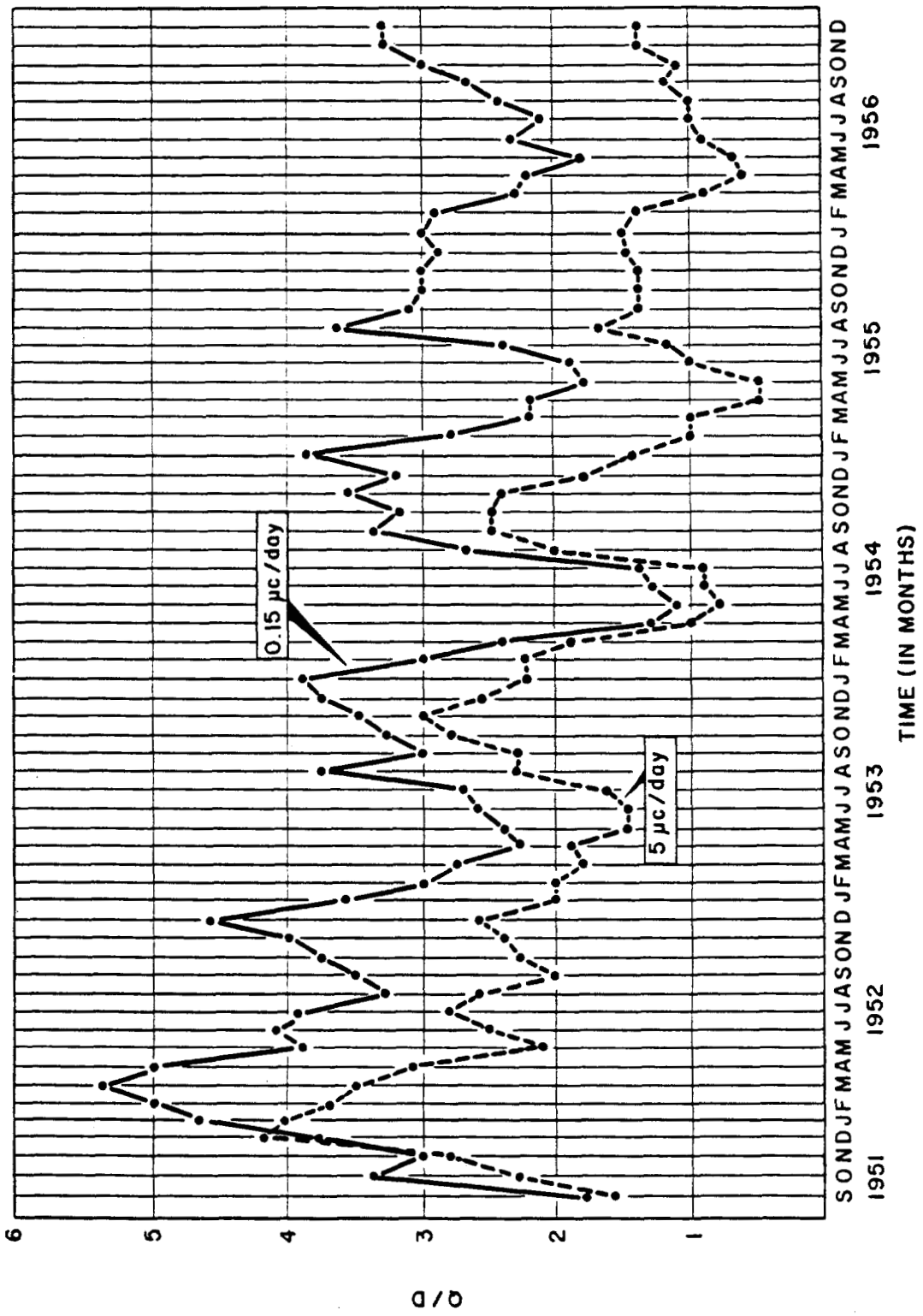

FIGURE 1
Variations of the Ratio Q/D (I^{131} in Thyroid/I^{131} Fed Daily) with Time
(Second and Third Year Offspring)

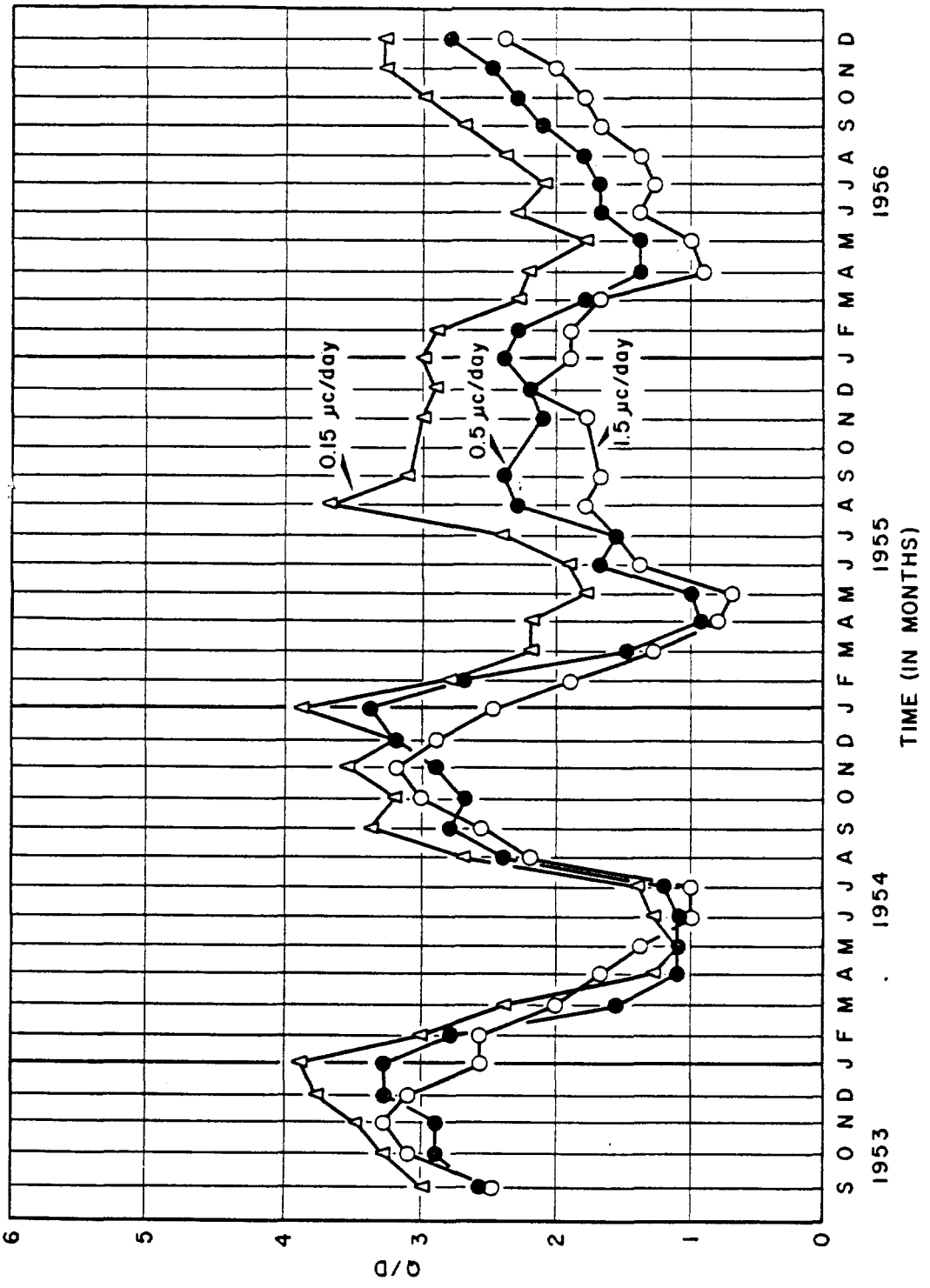

FIGURE 2
Variations of the Ratio Q/D (I^{131} in Thyroid/I^{131} Fed Daily) with Time
(Second and Third Year Offspring)

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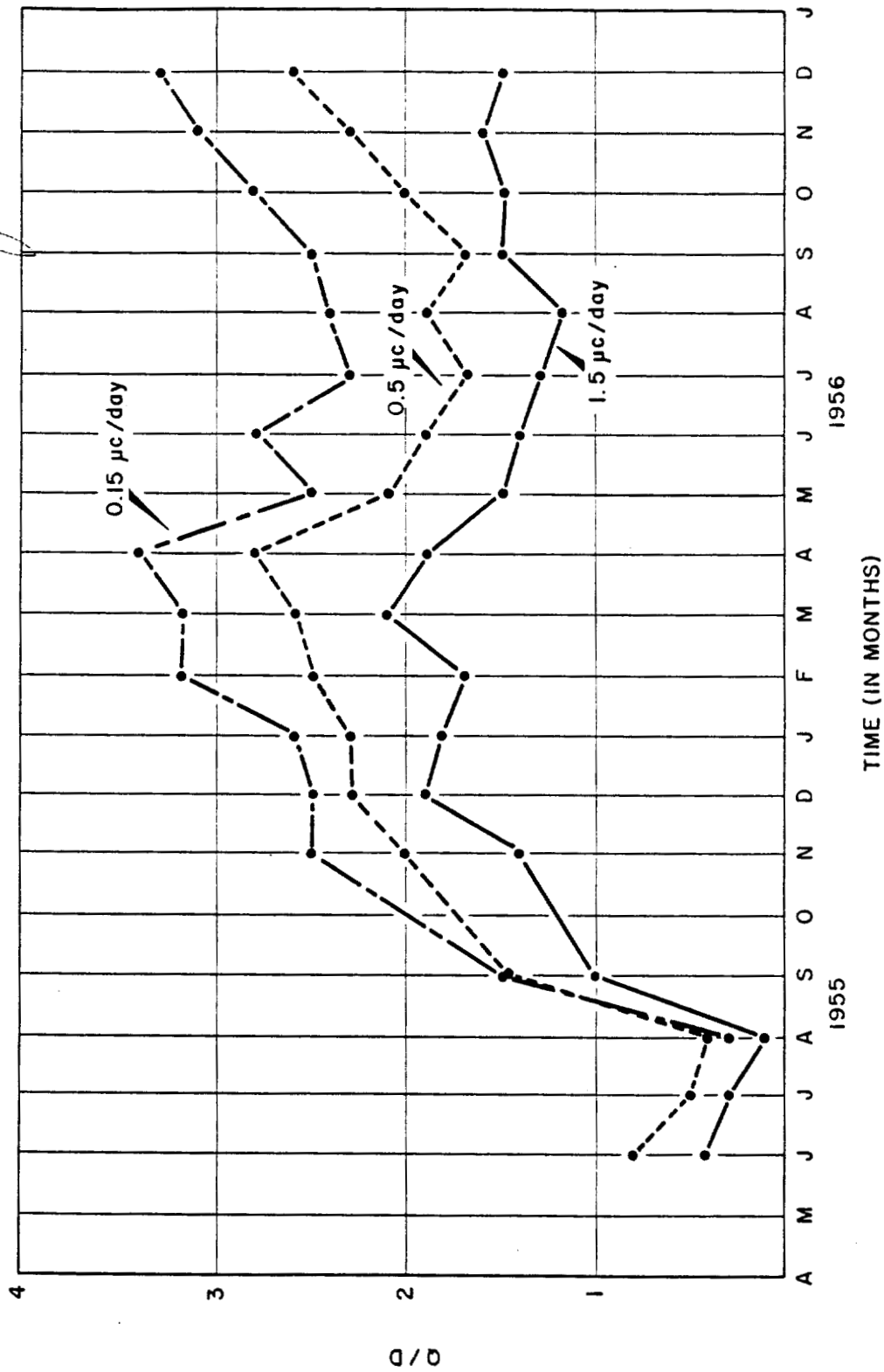

FIGURE 3
Variations of the Ratio Q/D (I^{131} in Thyroid/I^{131} Fed Daily) with Time
(1955 Offspring)

Blood Constituents (Mean Values) in Groups of 1951-1952 Offspring

Constituent	Control Group	5 μc/day group	1.5 μc/day group	0.5 μc/day group	0.15 μc/day group
PBI (μg/100 ml)	3.88 ± 0.30	1.16 ± 0.31	3.55 ± 0.47	2.98 ± 0.44	3.80 ± 0.38
Cholesterol (mg/100 ml)	84.2 ± 5.8	90.7 ±10.8	90.8 ±10.5	85.8 ± 6.2	77.4 ± 5.4
Inorganic phosphorus (mg/100 ml)	6.01 ± 1.02	5.58 ± 0.80	5.16 ± 0.47	5.97 ± 0.75	6.45 ± 0.71
Creatine (mg/100 ml)	0.93 ± 0.03	1.19 ± 0.13	0.79 ± 0.07	1.05 ± 0.09	1.03 ± 0.06
Packed-cell volume (%)	35.7 ± 1.5	36.8 ± 1.3	36.8 ± 1.4	37.3 ± 1.6	41.3 ± 1.3
Hemoglobin (g/100 ml)	12.05 ± 0.41	12.00 ± 0.41	12.39 ± 0.45	12.15 ± 0.31	13.25 ± 0.36
Leukocytes (cells x $10^3/mm^3$)	8.01 ± 0.53	7.89 ± 1.11	8.39 ± 0.86	8.64 ± 1.00	9.08 ± 0.59
Lymphocytes (cells x $10^3/mm^3$)	4.59 ± 0.28	4.67 ± 0.66	4.35 ± 0.25	5.32 ± 0.45	5.62 ± 0.39
Neutrophils (cells x $10^3/mm^3$)	3.18 ± 0.38	3.00 ± 0.55	3.77 ± 0.72	3.08 ± 0.65	3.16 ± 0.39

ACKNOWLEDGMENTS

The valuable assistance of M. E. Kerr and Experimental Farm Staff; John Herring, Radiochemistry Laboratory, Biological Analyses Operation; personnel of the Clinical Laboratory, Biological Analyses Operation; and N. L. Dockum, E. Jane Coleman and Glenda Vogt of the Histology Laboratory, Biological Analyses Operation is gratefully acknowledged.

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1086634

THYROID ADENOMAS IN SHEEP FED 5 μc I^{131}/DAY

S. Marks*, L. A. George, Jr.**, L. J. Seigneur*** and L. K. Bustad

The occurrence of multiple thyroid adenomas in four sheep administered a daily oral dose of 5 μc of I^{131} throughout their life span is described. Mild to moderate radiation damage of the thyroid parenchyma was observed in the affected glands.

The development of a fibrosarcoma in the thyroid region of a ewe 53 months old was described in an earlier report (1). The ewe was exposed to I^{131} by way of the fetal circulation, maternal milk, and, after weaning, daily oral administration of 5 μc of I^{131} throughout the duration of its life. Its dam had received a similar dose of 5 μc of I^{131} daily during pregnancy and lactation. Four additional members of this experimental group which were maintained on the same regimen were sacrificed during the past year at 68 months of age. They all showed the presence of thyroid adenomas, the findings in the thyroid glands of these animals being the subject of the present report.

Grossly, the thyroids of the affected sheep were smaller than normal. The thyroids showing tumor involvement averaged two grams, compared with nine grams in the 0.15 μc/day group. Only two of the affected thyroids exhibited gross lesions. The gland of one animal presented a single grayish-tan colored nodule that did not exceed 1 cm in greatest diameter, and in the other ewe the involved lobe had three small light gray colored nodules, none of which exceeded 4 mm in diameter.

Apart from the presence of adenomas, the principal pathologic feature was the presence of edema between the follicles in all glands (Figure 1). The degree of edema varied from mild to severe. Mild fibrosis was present in two of the four glands. A general reduction in the size of the follicles occurred in all glands.

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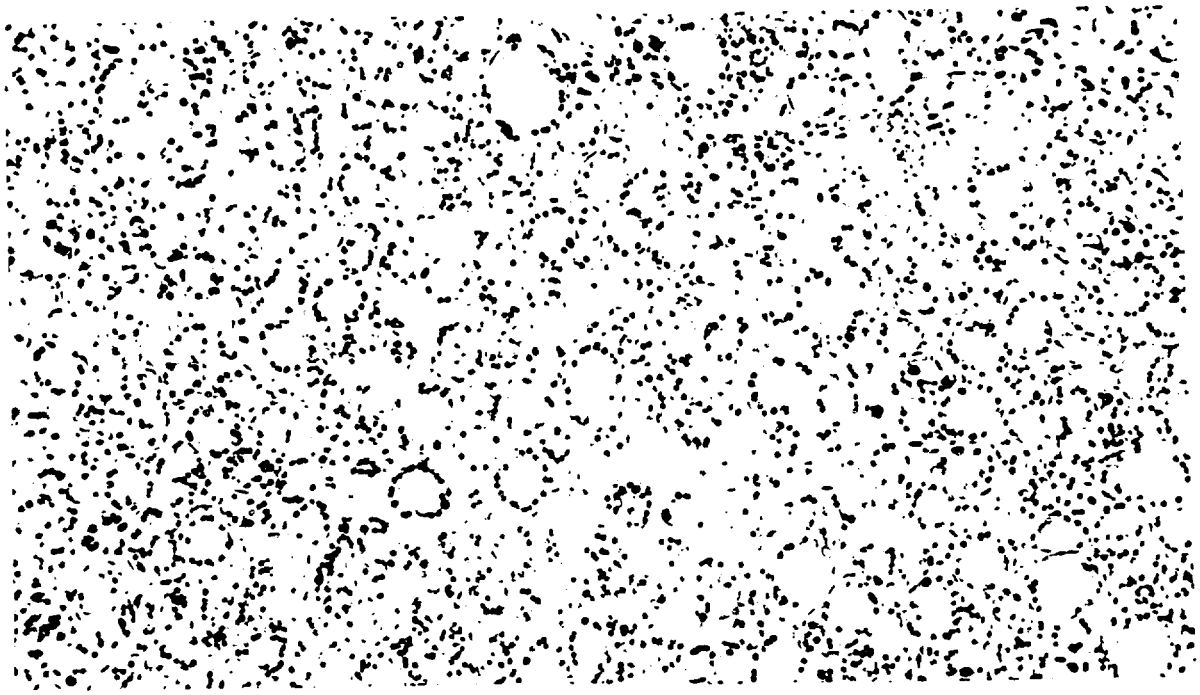

FIGURE 1

Microscopic Section of Thyroid Gland from Ewe Fed 5 μc/day for Over Five Years Showing Edema between Follicles. (115X)

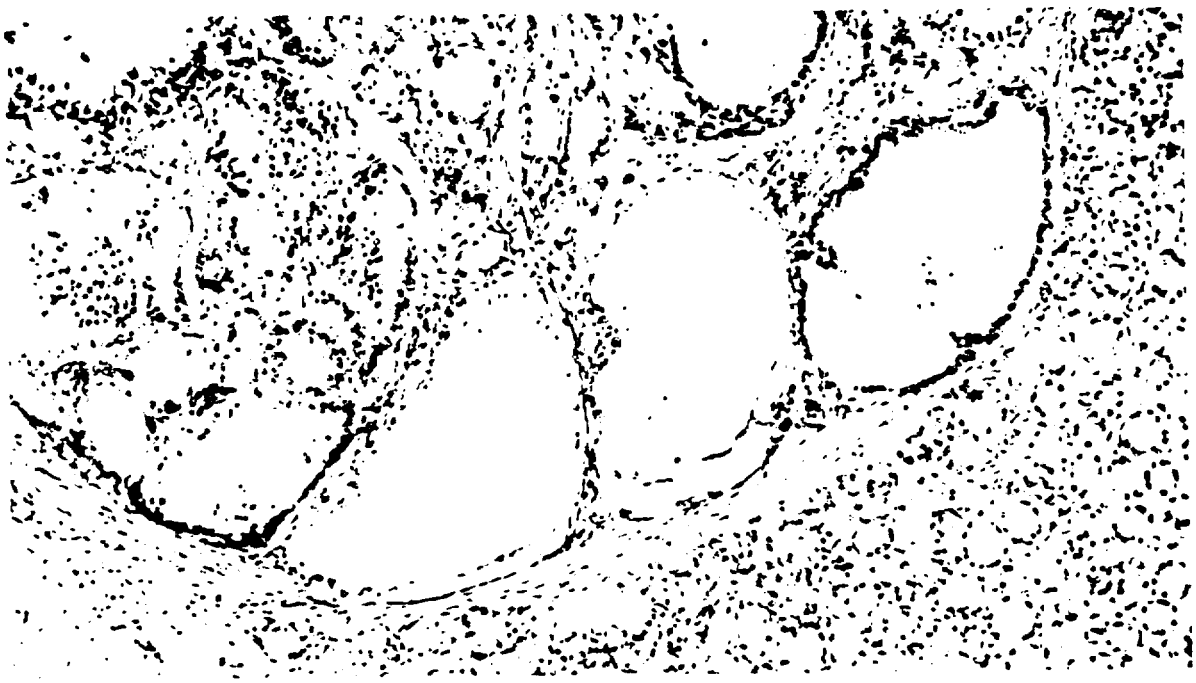

FIGURE 2

Microscopic Section of Thyroid Gland, from Ewe Fed 5 μc/day for Over Five Years, Showing Cross Section of Adenomas. Note Follicular and Tubular Pattern. (112X)

AEC-GE RICHLAND, WASH

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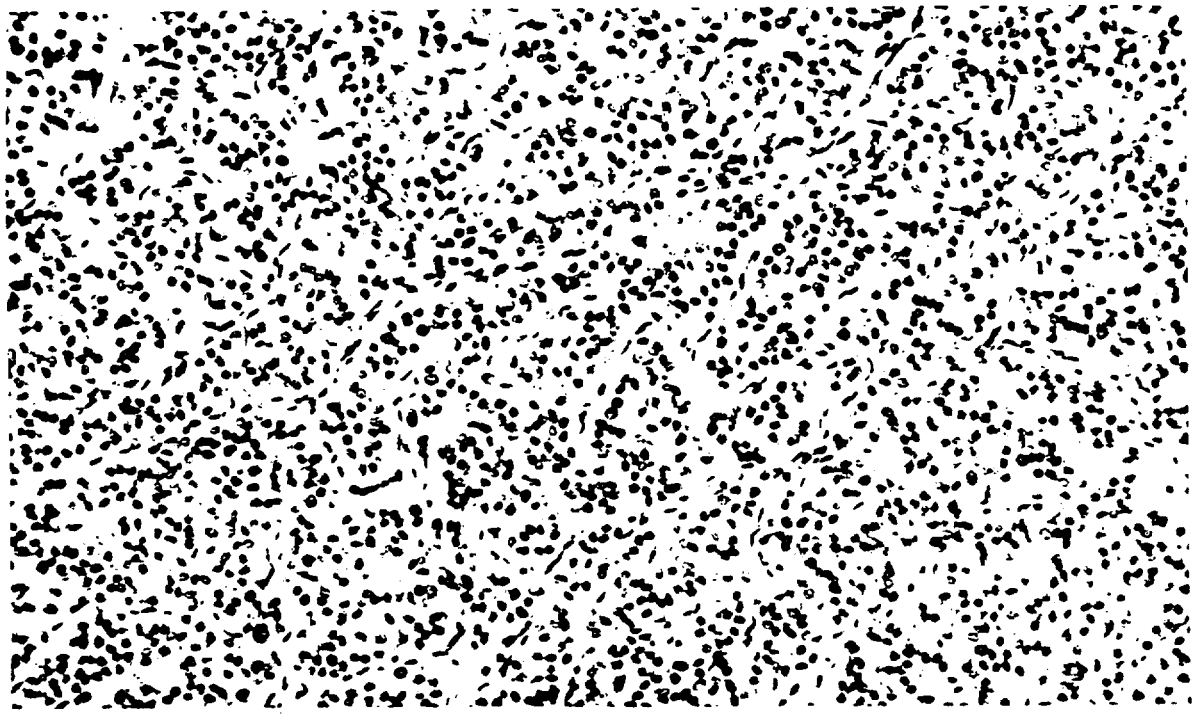

FIGURE 3

Cross Section of Thyroid Adenoma Showing Cord-like Pattern. (180X)

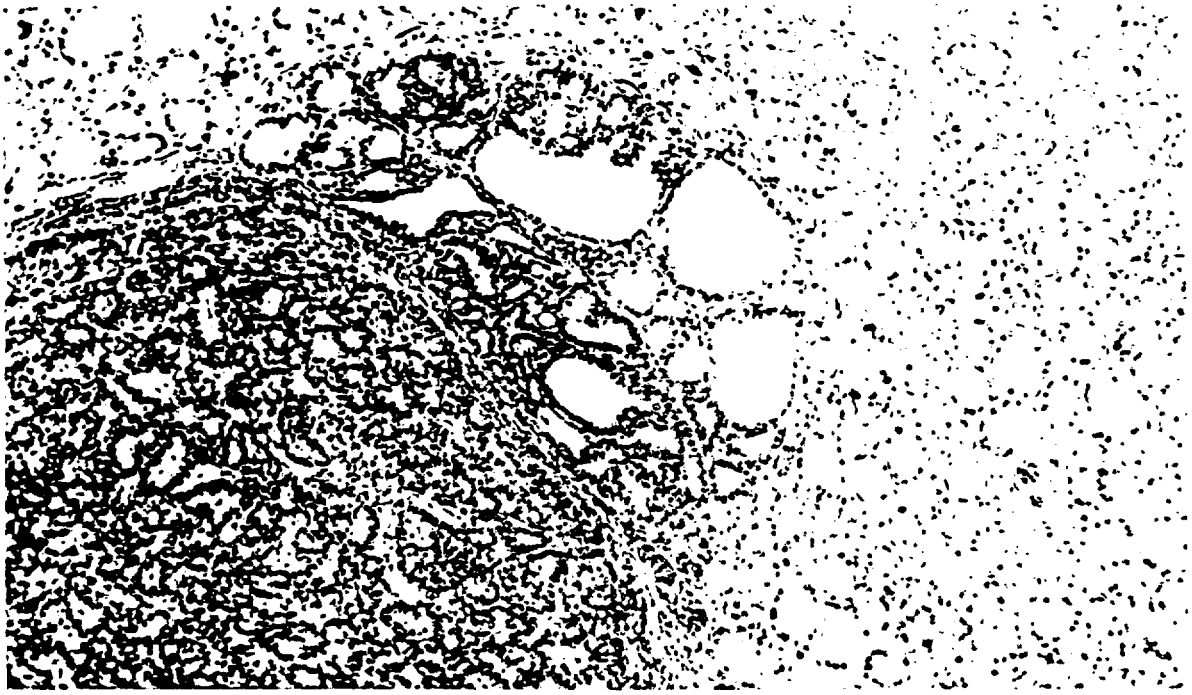

FIGURE 4

Cross Section of Thyroid Adenoma Showing Extension of Adenoma Beyond Confines of Capsule. (115X)

AEC-GE RICHLAND, WASH.

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The fact that all four glands in this group developed tumors and the multiplicity of the tumors suggests that the thyroid tissue had undergone a basic change favorable to tumor development. The absence of any such tumors in control material effectively rules out the assumption that the tumors might represent a natural event in the aging process. The combination of these observations with the previously described development of a fibrosarcoma in the fifth member of this experimental group permits the inference that I^{131} is tumorigenic under the conditions encountered in this experimental group. Those conditions are continuous exposure of sheep from fetal life until advanced age to quantities of I^{131} which cause mild to moderate damage to the thyroid gland over the indicated span.

ACKNOWLEDGMENT

The valuable assistance of V. G. Horstman, M. E. Kerr and staff of the Experimental Animal Farm and N. L. Dockum, E. Jane Coleman and Glenda S. Vogt of the Biological Analyses Operation is gratefully acknowledged.

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1086638

PROCEDURE FOR SURGICAL THYROIDECTOMY IN SHEEP

L. A. George, Jr.,* C. M. Barnes** and L. K. Bustad

This paper describes a procedure for surgical thyroidectomy in sheep, developed for the purpose of comparing the long-term physiological effects of surgical thyroidectomy with the effect of thyroid ablation by means of the administration of I^{131}.

REGIONAL ANATOMY

The thyroid gland of a normal sheep which has received adequate dietary iodine consists of two elliptical lobes, each approximately four to five centimeters long and one centimeter wide. Each lobe extends from about the second to the seventh tracheal ring. The lobes and the isthmus connecting their two caudal poles are firmly attached to the ventrolateral surface of the trachea. The isthmus varies considerably in size and shape but is never completely absent. It generally appears as a fleshy band approximately one centimeter wide extending between the two caudal poles. The dorsal border and cranial and caudal poles of the gland are contiguous with the thymus gland and in close association with the carotid sheath and structures contained therein. The ventral portion of the lateral surface of the thyroid is covered by the sternothyrohyoid muscle, the dorsal portion by the omohyoid muscle.

The vessels associated with the gland consist of the anterior and posterior thyroid arteries and their satellite veins. The anterior thyroid artery is a large branch of the common carotid artery which curves over the anterior end of the gland, into which it sends several branches. Smaller branches of the artery supply other structures in this region. The posterior thyroid artery, a small vessel which arises from the common carotid artery at a variable distance caudal to the anterior thyroid artery, also supplies

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small branches to adjacent structures.

The internal parathyroid glands are located in the parenchyma of the thyroid lobes and are removed with the thyroid gland. The paired external parathyroid glands are located in the loose connective tissue adjacent to the dorsal surface of the common carotid artery and caudal to the point where the occipital artery arises from the latter vessel. They are sufficiently distant from the surgical field to minimize the possibility of damaging them.

SURGICAL PREPARATION

Twenty-four hours before surgery, food and water are restricted and the operative area is clipped and washed. General anesthesia is obtained with pentobarbital sodium*, adminstered intravenously, 10 mg per kg of body weight. With the operating table adjusted to an angle of 20 degrees to the horizontal plane, the anesthetized animal is laid on its left side with the head at the higher level. The head and neck are then rotated from a lateral to a dorsal recumbent position, the chin directed straight forward, and the neck extended and arched slightly backward, but avoiding respiratory embarrassment. The operative area is again clipped, scrubbed, disinfected with isopropyl alcohol and amply draped.

SURGICAL TECHNIQUE

The inital incision is in the midline, extending caudally for about 14 cm from the cricoid cartilage. The skin flaps, including the superficial fascia, are reflected laterally by blunt dissection, exposing the paired sternothyrohyoid and omohyoid muscles (Figure 1). These muscles and the deep layer of fascia are then separated at the midline and retracted laterally with a Volkmann retractor, exposing the thyroid gland (Figure 2). Good exposure is essential to permit unobstructed manipulation and double ligation of the major vessels.

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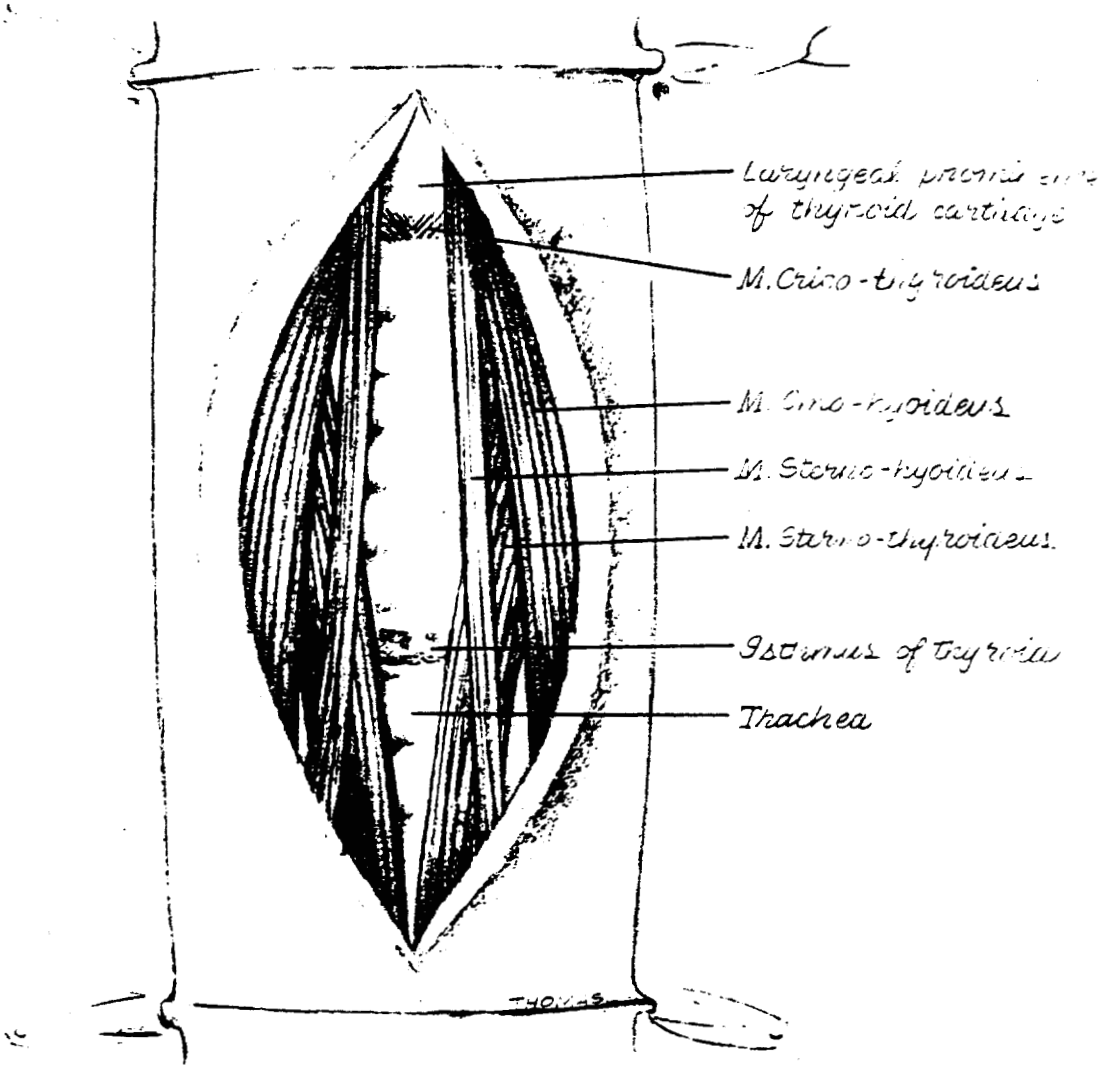

FIGURE 1

Retraction of the Skin and Superficial Fascia Reveals the Ventral Surface of the Trachea and Paired Sterno-thyrohyoid and Omohyoid Muscles

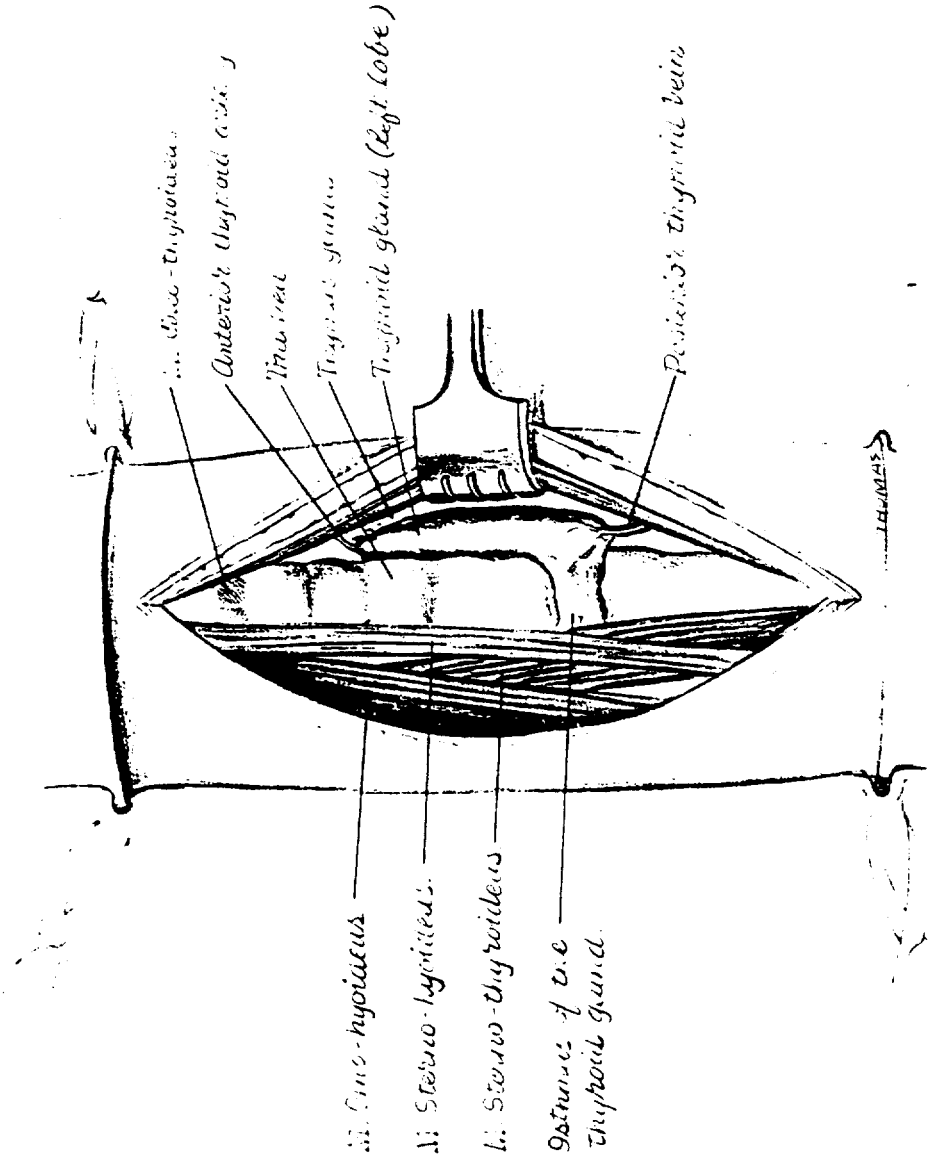

FIGURE 2
Retraction of the Skin and Muscles
on the Left Side Reveals the Isthmus and Left Lobe of the Thyroid Gland

AEC-GE RICHLAND, WASH.

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Excision is begun by isolating the caudal thyroid vessels and ligating them with braided silk ligatures. The cranial thyroid vessels are isolated and similarly ligated (Figure 3). The cranial thyroid vessels are then divided. Removal of the intact gland is started at either cranial pole. The medial surface of the lobe is gently freed from the trachea by blunt dissection. The body of the lobe is grasped by the fingers with sterile gauze, gently retracted, and small, sharp-pointed hemostats are inserted from the front, just ahead of the line of incision which is then made adjacent to the dorsal border of the lobe. In this way no special attempt is made to clamp the smaller individual vessels which enter the gland along this border. Instead, the tissue is clamped and ligated proximal to the hemostats. Reasonable care must be taken to avoid damage to the structures contained in the carotid sheath. Following ligation of each small mass of tissue, division between the ligature and hemostat is accomplished by means of thermocautery to save time and minimize hemorrhage. When the doubly-ligated caudal thyroid vessels are reached they are divided and the lobe rotated medially. The same procedure is followed in freeing the second lobe. When both lobes are free the remaining attachment of the isthmus to the trachea is separated by blunt dissection and the intact gland is removed.

When the surgical area has been fully inspected for remnants of thyroid tissue, particularly in the region of the cranial and caudal poles, and all bleeding has been controlled, the incision is closed. Interrupted sutures of fine braided silk are used to approximate the sternothyrohyoid muscles, with stainless steel mattress sutures used in the subcutaneous tissue and skin.

Recovery from the anesthetic usually requires one to two hours. Six hours after complete recovery from the anesthetic, the animal is given limited amounts of feed and water. Skin sutures are removed on the tenth post-operative day.

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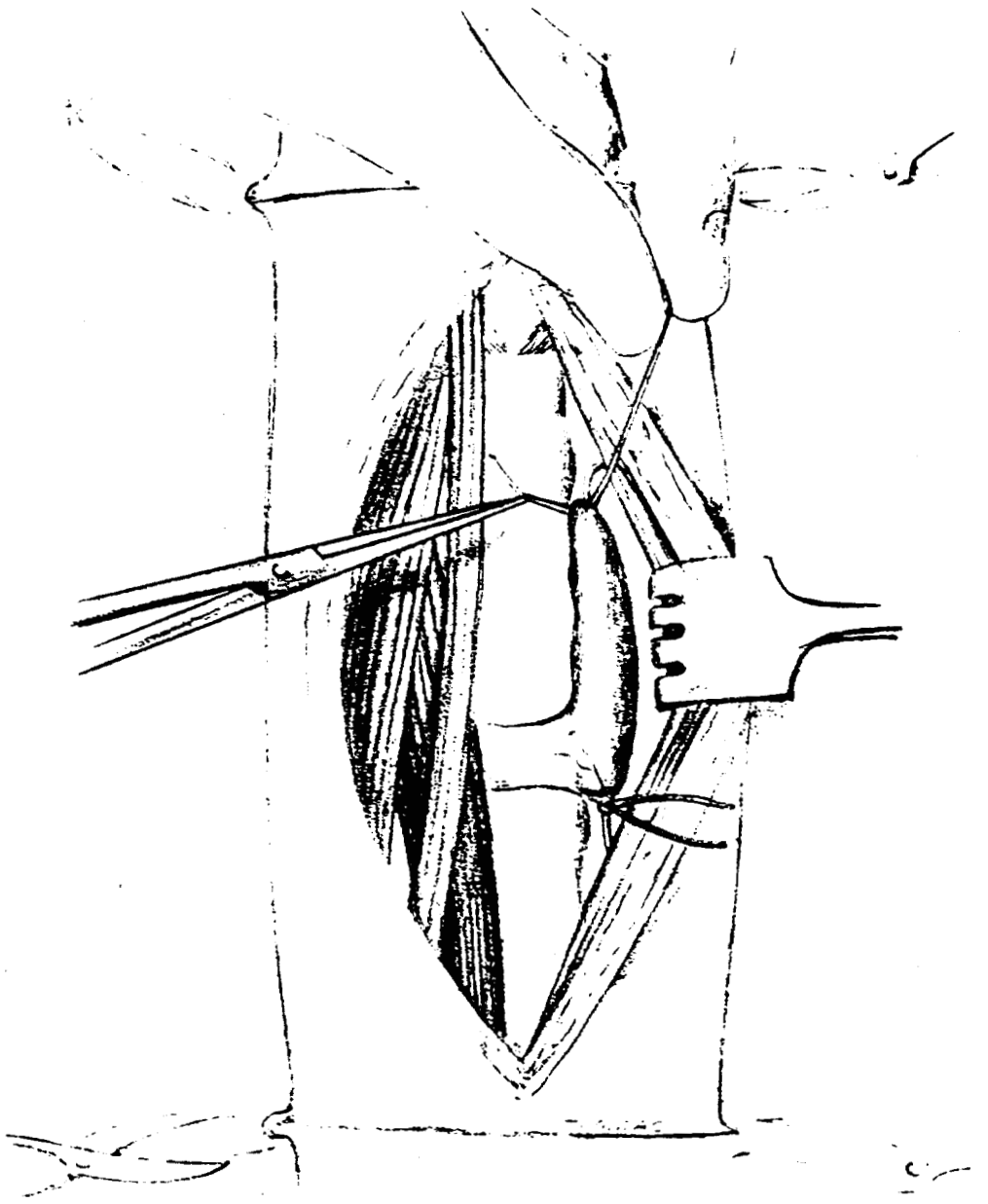

FIGURE 3

The Vessels Supplying the Left Lobe are Isolated and Ligated

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BIOLOGICAL EFFECTS OF I^{131} IN PIGS. I. NUTRITION LEVEL AS A FACTOR IN THYROIDAL - I^{131} METABOLISM

L. J. Seigneur*, P. L. Hackett, and L. K. Bustad

Differences in thyroid metabolism in pigs maintained on two levels of nutrition are described in conjunction with a study to determine the minimal daily I^{131} intake that will cause thyroid damage. Amounts of thyroidal I^{131} were higher in the pigs on full-feed than in those on 70 per cent of full-feed.

The long-term study of the biological effects of I^{131} in pigs is an extension of previous work on sheep (1). Preliminary investigations with pigs were limited to determining the reliability of a two-probed scintillation detector for accurate in vivo measurement, typical I^{131} uptake and excretion patterns, and methods of pig restraint (2, 3). Pigs which had been maintained on full-feed (high-plane) and 70 per cent of full-feed (low-plane) were obtained from the State College of Washington.

METHODS

Each of four high-plane and four low-plane gilts averaging 17 months of age was fed 45 μc of I^{131} on two separate occasions before being included in the chronic I^{131} study. The tracer studies were performed in order to determine the time of maximal thyroidal uptake and the effective half-life of I^{131}. For the chronic study, twenty purebred Palouse gilts were divided into five groups according to plane of nutrition and level of I^{131} fed daily (Table 1). The respective nutritional levels had been maintained for several generations.

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TABLE 1

Experimental Design of Chronic Study

Number in group	Average age (mo.)	Nutritional plane	μc I^{131} fed daily
4	17	High	45
4	17	Low	45
4	10	High	5
4	10	Low	5
4	10	Low	0.5

All pigs were group-fed a prepared ration, low-plane pigs receiving 70 per cent by weight of the daily allowance for high-plane pigs. Each new shipment of prepared ration, considered marginally goitrogenic due to low iodine content, was analyzed for stable iodine. The low-plane pigs received individual daily supplements of stable iodine in order to equalize the iodine content of their diet and that of the high-plane pigs.

The second tracer and chronic I^{131} studies were initiated after the thyroidal I^{131} concentrations had dropped below one microcurie. In the tracer studies, the thyroids were monitored at six-hour intervals for the first 48 hours and daily thereafter, using the specially designed pig thyroid monitor shown in Figure 1 (3). The thyroids were monitored daily in the chronic I^{131} study until equilibrium was reached and twice each week thereafter. Body background was determined at each monitoring by enveloping the thyroid region with a two-section, detectable, one-half-inch thick lead collar capable of shielding out 99 per cent of dectable gamma emission (Figure 1). Open counts (no thyroid shielding) minus body background provided net counts attributed to I^{131} in the thyroid.

Two blood samples were obtained during the second tracer study and four were collected in the first 50 days of the chronic I^{131} study. Determinations

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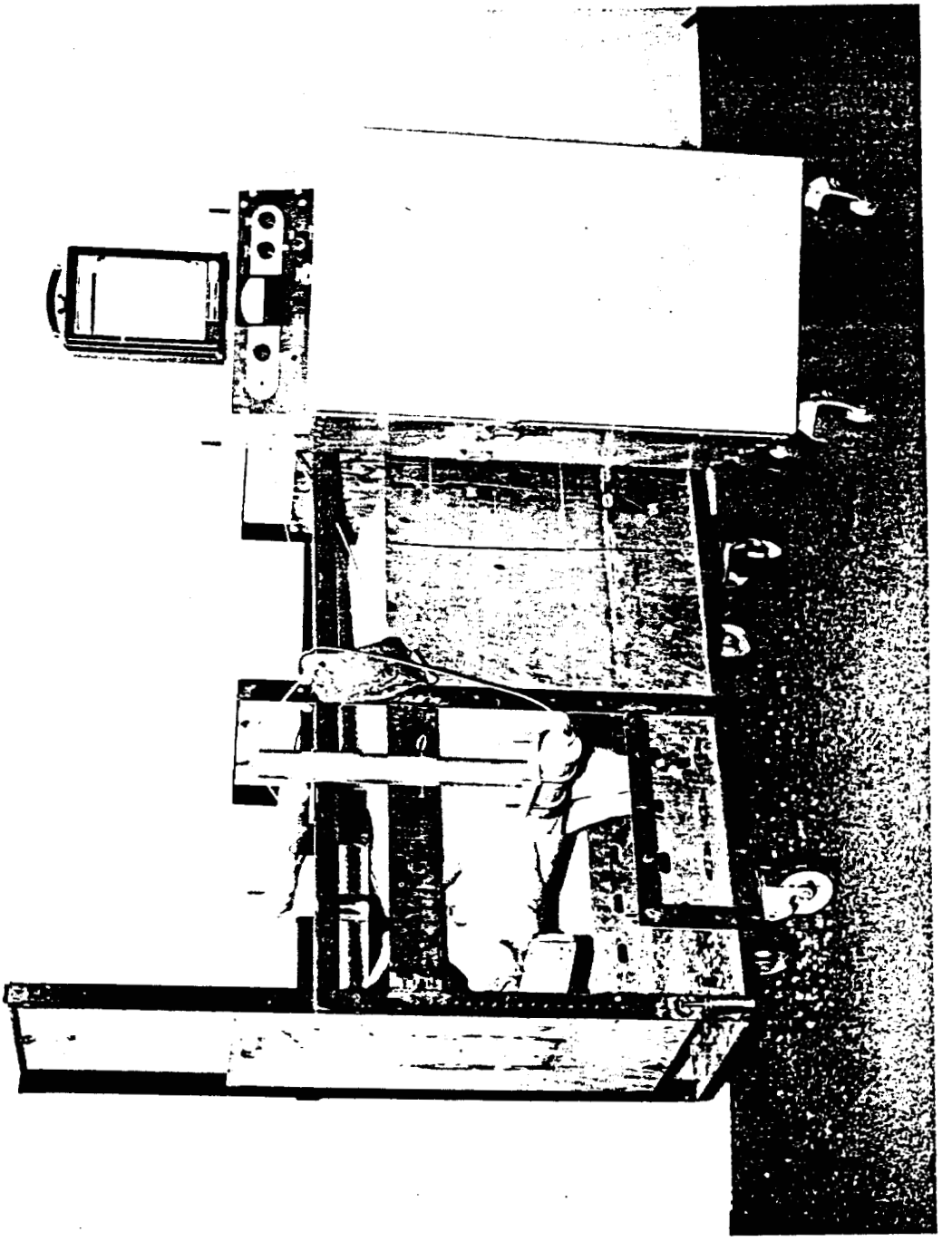

FIGURE 1

Monitoring I^{131} in Thyroid of Pig Using Two-Probe Scintillating Detector.

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of PBI^{131} and I^{131} content of the serum were made in order to calculate the serum conversion ratios (PBI^{131}/total I^{131}).

RESULTS AND DISCUSSION

Tracer I^{131} Uptake and Turnover

Tracer study comparison of the two nutritional levels showed no significant time differences in reaching maximum I^{131} uptake in the thyroid nor in the effective thyroidal I^{131} half-life. The average interval until thyroidal I^{131} attained maximum uptake was between 20 and 30 hours after feeding, and the effective half-life was 6 days. Maximum absorption coefficients (I^{131} in the thyroid/total I^{131} fed) were greater in the high-plane pigs (Table 2) and thyroidal I^{131} remained higher throughout the period of the study (Figure 2). This may be due to larger thyroids in the high-plane pigs, a factor that will not be known until sacrifice. Lower conversion ratios were evidenced in the high-plane pigs on the second day only (Table 2).

TABLE 2

Tracer I^{131} Absorption and Turnover

Tracer study	Nutritional plane	Maximum absorption coefficient	Serum I^{131}		Conversion ratio
			Days after feeding	$\mu c/ml \times 10^{-5}$	
I	High	0.31			
	Low	0.25			
II	High	0.33	2	2.3	0.4
			5	1.3	0.8
	Low	0.28	2	4.0	0.6
			5	2.0	0.9

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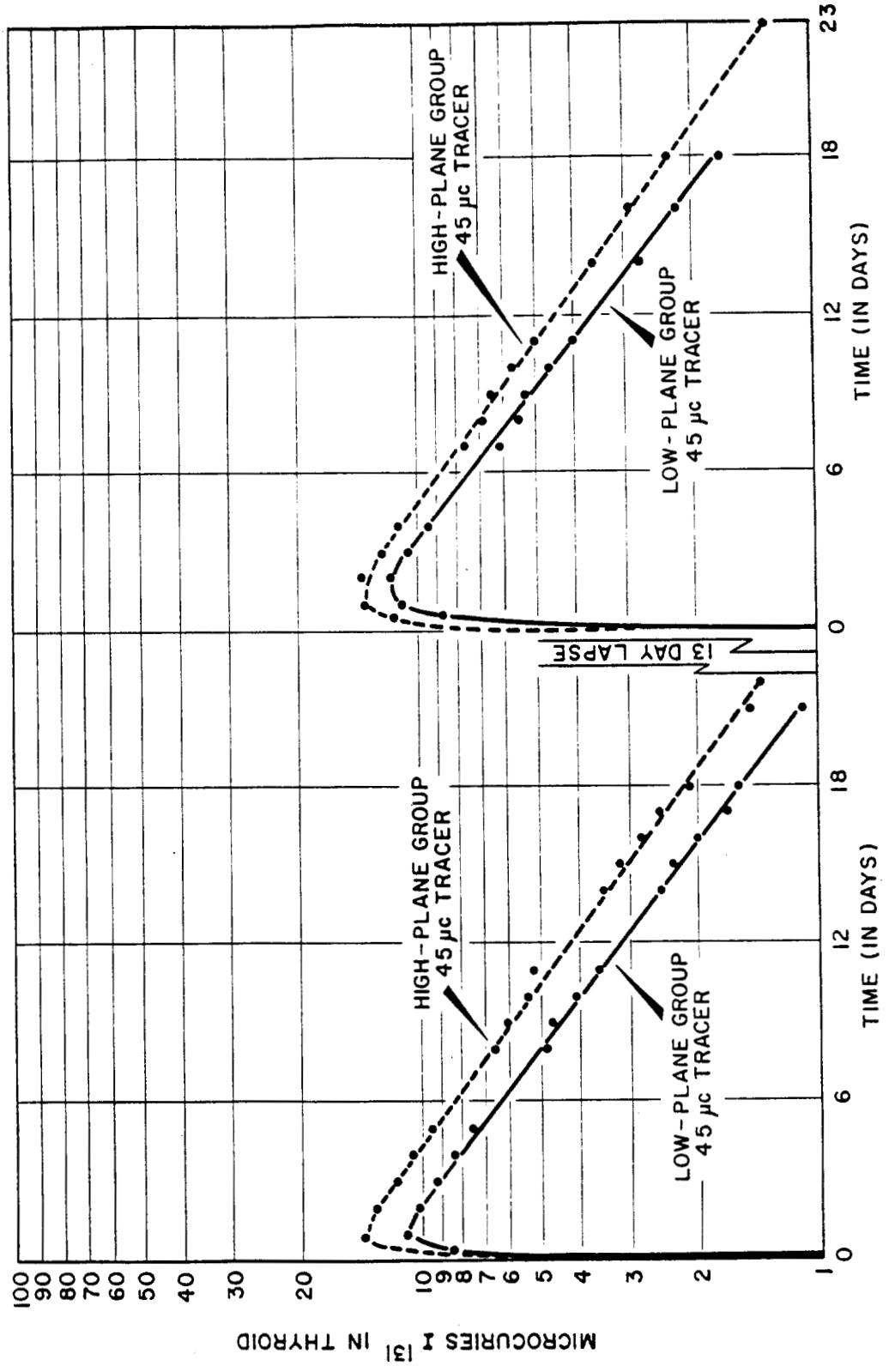

FIGURE 2

Thyroidal I^{131} Uptake and Decay of High and Low-Plane Pigs in Tracer Studies.

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Chronic I^{131} Uptake and Turnover

In the chronic I^{131} study, the average time required to reach thyroidal I^{131} equilibrium after first I^{131} fed was 21 days and appeared to be independent of the level of nutrition. As in tracer observations, the full-fed pigs showed higher values at thyroidal I^{131} equilibrium (Figure 3). Group averages of Q/D (I^{131} in the thyroid at equilibrium/I^{131} fed daily) and serum I^{131} conversion ratios are shown in Table 3. The high serum I^{131} value in the 5 μc/day full-fed pigs before equilibrium (19th Day) was due to an excessively high I^{131} value for one pig. In both tracer and chronic studies the serum I^{131} values appeared to be slightly greater in the low-plane pigs although the difference was not significant. This may be attributed to the small number of animals in the experiment or to the fact that the pigs were group-fed rather than individually fed.

TABLE 3

Chronic I^{131} Uptake and Turnover

μc I^{131} fed/day	Nutritional plane	Q/D at equilibrium	Serum I^{131}		
			Days after feeding	μc/ml x 10^{-5}	Conversion ratio
5	High	2.1	19	47.6	0.9
			34	26.1	0.8
			40	25.2	0.8
			49	35.0	0.8
	Low	1.3	19	35.7	0.8
			34	31.4	0.7
			40	30.8	0.8
			49	37.4	0.8
45	High	2.1	22	3.4	0.7
			36	3.9	0.9
			41	3.0	0.8
			49	3.9	0.8
	Low	1.5	22	7.8	0.8
			36	7.8	0.9
			41	4.2	0.9
			49	5.3	0.8

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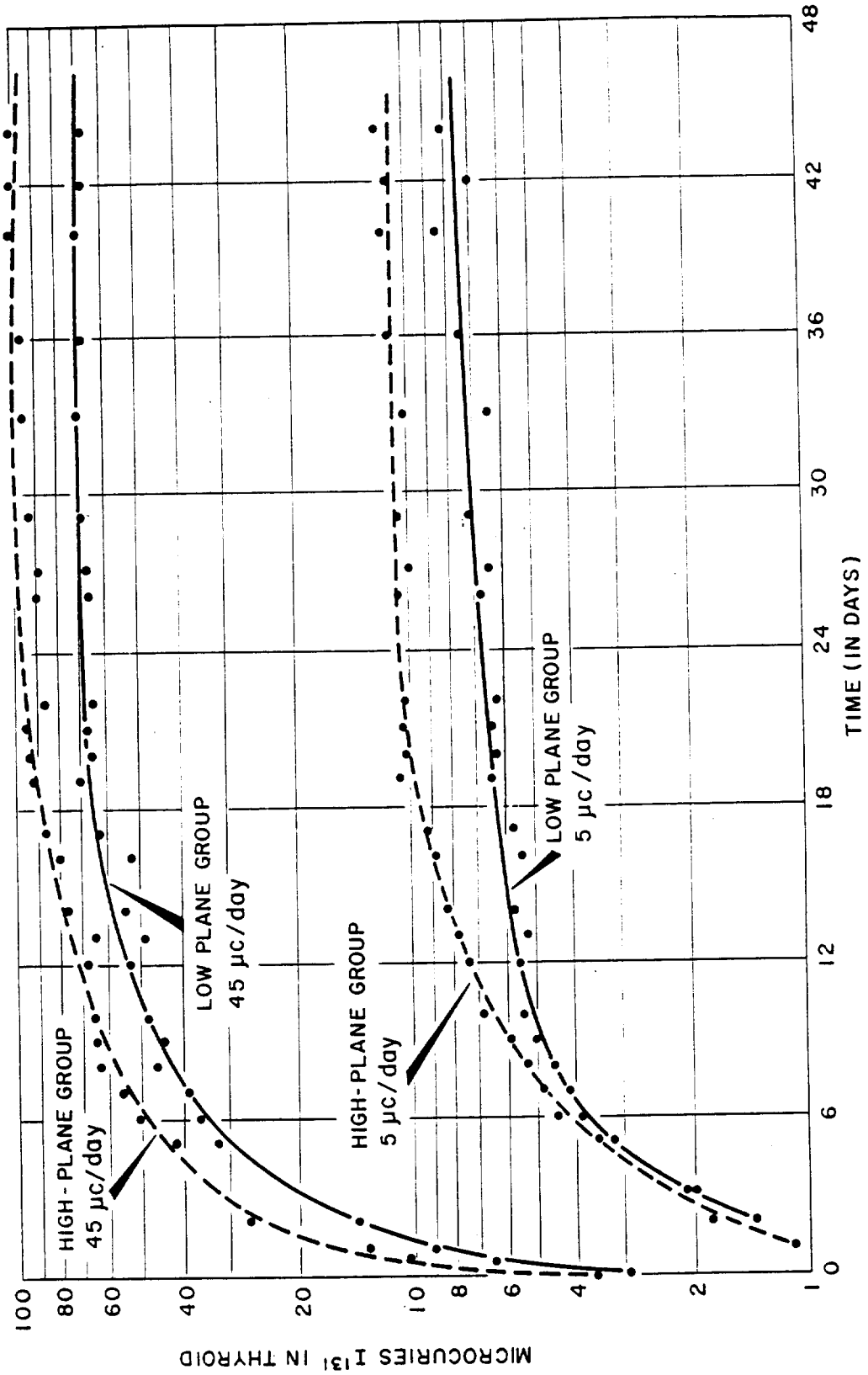

FIGURE 3

Thyroidal I^{131} Concentrations of High and Low-Plane Pigs in Chronic Study.

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ACKNOWLEDGMENTS

The valuable consultation services of Dr. M. E. Ensminger, Chairman of the Department of Animal Husbandry, State College of Washington, and the technical assistance of V. G. Horstman, M. E. Kerr, the Experimental Animal Farm attendants and Biological Analyses Operation personnel are gratefully acknowledged. The pigs were obtained from the project "A Fundamental Study of the Relationship of Nutrition to Improvement of Animals for Meat Production through Breeding," conducted by the Department of Animal Husbandry, State College of Washington.

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Leukocyte values for the high-plane pigs were slightly lower, but not more so than the expected difference between any two groups of animals. A decreasing trend in lymphocytes occurred as both group of animals matured. In January, 1956 the erythrocyte, packed-cell volume, and hemoglobin values in the high-plane pigs were significantly lower than those of the low-plane.

PBI values decreased and creatinine increased after the spring of 1956. This may be due to either season or age. Creatinine and protein levels in the high-plane pigs were slightly lower over the experimental period. Serum phosphorus and alkaline phosphatase decreased with time, especially in the low-plane pigs.

Since a decrease in lymphocytes and PBI, and an increase in creatinine follow thyroid ablation in sheep (3), the change in these constituents in pigs either from maturation or seasonal effects, is particularly noteworthy. Alterations in serum cholesterol would be difficult to detect as considerable variation was noted in this constituent.

ACKNOWLEDGEMENTS

We wish to thank the Biological Analyses and Experimental Animal Farm operations personnel for their technical assistance.

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TRIPLE RADIATION ASSAULT ON DEBILITATED LAMBS

L. A. George, Jr.*, P. L. Hackett, and L. K. Bustad

Debilitated lambs were exposed to a triple radiation assault consisting of 10, 50, or 100 r of whole-body x irradiation, oral administration of 200 or 650 μc I^{131}, and 2,000 or 16,000 rad of beta-irradiation to localized areas of skin. Discoloration and scarring of the beta-irradiated lesions of the skin followed exposure to 16,000 rad. Marked leukocyte depression was observed only in the group exposed to 50 or 100 r. Six months after the radiation assault the animals were alert and in good physical condition.

Although extensive research work has been performed on large animals regarding the effects of the individual modes of radiation exposure encountered in fallout, no data are available with respect to the effects of a multiple radiation assault. In view of the lack of such information, an experiment was performed involving the exposure of sheep to bilateral whole-body x irradiation, oral ingestion of I^{131}, and beta-irradiation of skin. These experimental conditions are representative of an external gamma source, an external beta emitter, and an internal beta emitter. In addition, the experimental sheep were maintained on a low nutritional plane, since it has been reported that an animal's susceptibility to whole-body irradiation may be modified by a deficient diet (1, 2).

METHODS

Twenty-eight weaned purebred Suffolk lambs of both sexes comprised the experimental group. Table 1 shows the distribution of the animals and other pertinent features of the experimental design. Five to six weeks before exposure to the triple radiation assault, twenty-two of the test animals were established on a low nutrition diet. At the time of exposure their body

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TABLE 1

Experimental Design

		Radiation assault		
Number of animals	Nutritional level	Whole-body dose	Beta skin dose	I^{131} Oral dose
6	normal	none	none	none
6	low	none	none	none
4	low	100 r*	2,000 or 16,000 rad	200 μc (1,000 rad)
8	low	50 r**	16,000 rad	650 μc (3,000 rad)
4	low	10 r*	2,000 or 16,000 rad	700 μc (3,500 rad)

* Exposure Conditions: 250 kv, 30 ma
Filtration: 0.25 mm Cu and 1.0 mm Al
Distance to midline of animal: 183 cm
Dose Rate: 12r/min.
Half Value Layer: 0.40 mm Cu
Effective energy (calculated in air): 88 kev

** Exposure Conditions: 250 kv, 30 ma
Filtration: 2.5 mm Cu and 1.0 mm Al
Distance to midline of animal: 183 cm
Dose Rate: 4 r/min.
Half Value Layer: 3.5 mm Cu
Effective Energy (calculated in air): 145 kev

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weight had become constant at a lower level than normal (Figure 1) and all of the animals showed signs of mild weakness and depression. The body weights of the 10 r group did not differ significantly from those indicated for the 100 r group.

The sequence of exposure to the triple radiation assault was as follows: The whole-body x-ray dose was delivered first, followed in twelve hours by oral ingestion of 200 to 700 μc of I^{131} contained in a food pellet. Within 48 hours after the whole-body x-ray exposure, two localized areas of skin on each side of the lateral back region were exposed to the beta radiations from a Sr^{90} source, measuring 4.5 cm in diameter and delivering a surface dose rate of approximately 8,700 rad per hour. Immobilization of the animals for the x ray and beta radiation exposures was accomplished by suspending the animals in a canvas sling. The method of preparing the skin for the beta exposures and a description of the exposure device were previously reported (3).

The animals were examined daily throughout the post-irradiation phase of the experiment. Blood samples were taken from control and test animals every other day for the first week and at three to four-day intervals for an additional four months. Hematologic procedures included total leukocyte and differential counts.

RESULTS AND DISCUSSION

The results of this experiment are divided into three categories: the clinical appearance and behavior of the animals, the gross appearance of the beta skin lesions, and the hematologic results.

Within 24 hours after exposure to 50 or 100 rad of total body x-irradiation, four of the animals appeared somewhat depressed for three to four days, followed by a more alert appearance and an increased interest in their surroundings. The appetite of all the animals remained good during this period. After this initial manifestation had passed, no further adverse signs attributable to the whole-body exposure were observed.

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The gross appearance of skin lesions following beta-irradiation is summarized in Table 2. The only observable effects in the areas of skin exposed to 2,000 rad were a transient cessation of wool growth and a marked change in color of the skin. This color change, which has remained for over 15 months is attributed to an increase in pigment, and appeared as a gray discoloration of the skin. Apart from these features, the skin appeared normal. The areas exposed to 16,000 rad underwent a much more profound reaction characterized by immediate swelling, followed by a gradual process of dry desquamation and eventual healing. The healed lesions were slightly distorted in shape, presumably because of natural changes in skin tension with growth of the animal and an apparent increased amount of fibrous tissue beneath and adjacent to the lesion. This was evidenced by stiffness and loss of normal resilience of the skin and subcutaneous tissue in these regions. None of the exposure sites became infected during the post-exposure period.

The lambs exposed to 50 or 100 r of whole-body x ray show a marked leukocyte depression within 12 hours after exposure. This is attributed to a severe drop in the lymphocyte count, as seen in Figure 2, and a less severe drop in the neutrophil count. Variations occurring in lymphocyte values in both the control groups and the 10 r group are not considered to be significant under the conditions of this experiment.

Six months after exposure all animals exhibited a normal blood picture, were alert, and in good physical condition. At that time the exposed animals weighed approximately the same as the control animals maintained on full-feed, although they had weighed considerably less than the control animals at the start of the experiment (Figure 1). The control and exposed animals were indistinguishable except for the scars on those animals exposed to 16,000 rad of beta-radiation. Irradiated ewes that were serviced by irradiated rams following all radiation exposure gave birth to normal lambs.

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TABLE 2

Gross Pathologic Changes Following Beta-Irradiation of Sheep Skin

Days after exposure	2,000 rad		16,000 rad	
	Wool	Skin	Wool	Skin
0	No change.	No change.	No change.	Some areas swollen when exposure source removed.
1-3	Wool appeared matted. Complete cessation of growth	No change.	Complete cessation of growth. Wool appeared matted.	All areas underwent severe swelling.
8-10	Wool was more matted.	Skin became dry.	Wool was more matted and began to fall out.	No swelling, skin was dry and bluish-pink in denuded areas. Areas were sensitive to touch.
15-17	No change. Wool did not fall out.	Skin was sensitive but not changed in appearance.	Wool fell out in all areas.	Skin was grayish-blue, dry, non-resilient, and less sensitive.
28	No change.	No change in appearance. Areas were not sensitive to touch.	Complete cessation of wool growth	Skin was grayish-blue, dry, non-resilient, and not sensitive to touch.
36	Wool regrowth began.	Mild hyper-pigmentation of epithelium.	Complete cessation of wool growth.	New, thin, pink epithelium was growing beneath a stiff epithelial disc.
51	Wool growth was normal.	Skin was light to dark gray but normal in texture.	Same.	Repair process continued beneath sloughing epithelial disc. Areas were hypersensitive.

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TABLE 2 (Contd.)

Days after exposure	2,000 rad		16,000 rad	
	Wool	Skin	Wool	Skin
71	Same.	Same.	Same.	New nonpigmented epithelium had completely covered most of the exposed areas.
90	Same.	Same.	No regeneration of wool fibers in exposed areas.	Repair of exposed areas was complete. Borders were sharply demarcated by a black pigmented zone.

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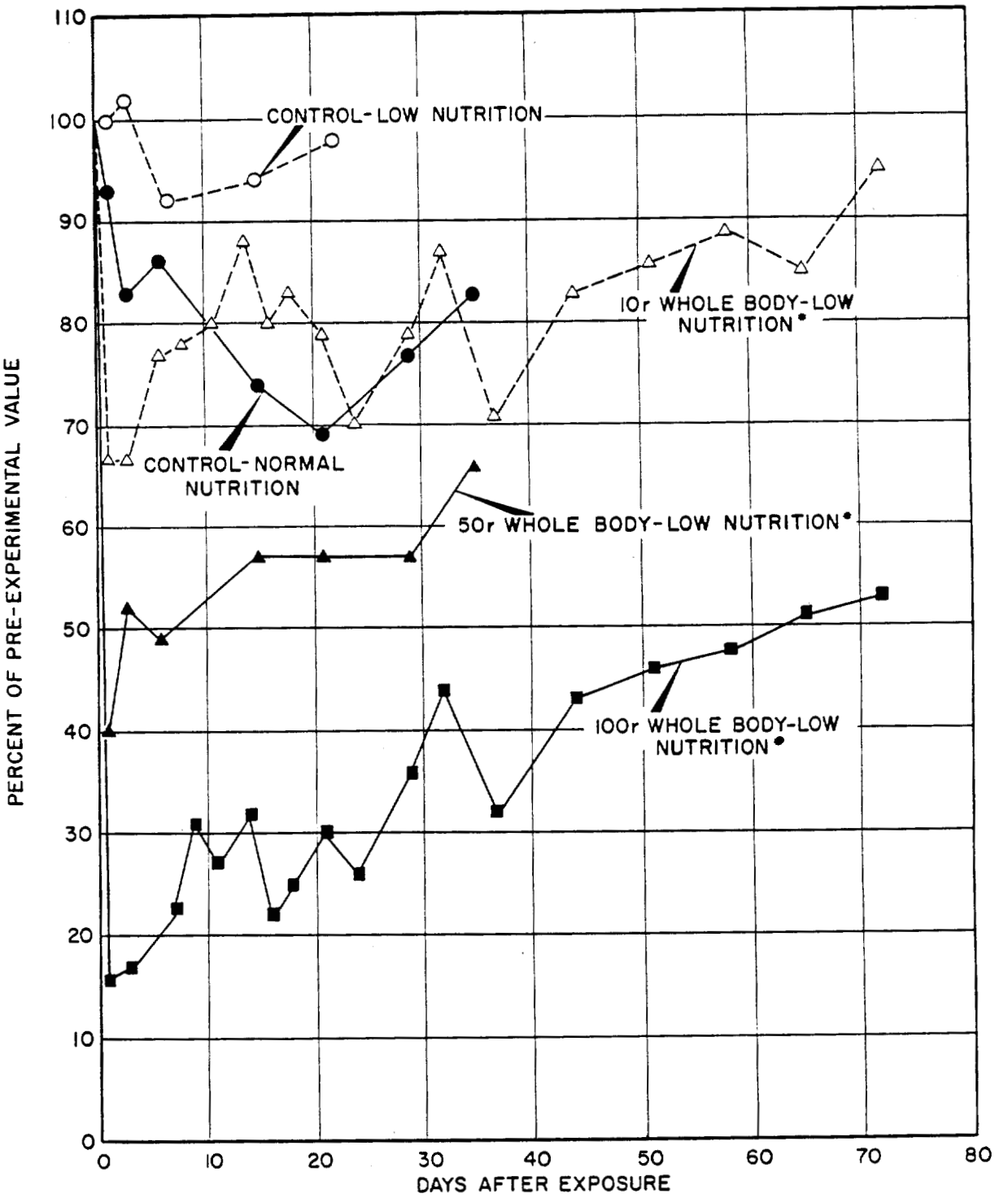

FIGURE 2

Per Cent of Pre-experimental Lymphocyte Values in Control and Exposed Animals.

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LEUKOCYTIC RESPONSE IN LAMBS EXPOSED TO WHOLE-BODY X IRRADIATION

L. A. George, Jr.*, Patricia L. Hackett, and L. K. Bustad

Six animals exposed to 30 r exhibited a significant depression in lymphocytes but showed no other signs of damage. A comparable group of animals exposed to 15 r resembled the control animals in all respects.

In the preceding paper an appreciable difference in the early leukocytic response of the animals following exposure to the different whole body dose levels is reported. Compared with pre-irradiation levels the response ranged from no significant depression at the 10 r level to about 50 per cent depression at the 50 r level, and a 60 to 70 per cent depression at the 100 r level.

This observation stimulated interest relative to the dose level at which the first perceptible leukocytic depression would occur. The study reported here presents results obtained by exposing young male lambs to bilateral whole-body x-ray exposures of 15 to 30 r.

METHODS

Eighteen young male nursing Suffolk lambs weighing between 40 and 55 kg each were divided into three groups and irradiated under the conditions summarized in Table 1. The animals were immobilized during the exposures by being placed in a canvas sling which was suspended in the field of the x-ray generator (General Electric-Maxitron). The total dose was divided equally between the right and left sides of the animals. Control animals were similarly immobilized and suspended in the x-ray generator facility but were not irradiated.

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Three blood samples were obtained from each of the 18 animals during the week prior to exposure to obtain the pre-treatment data. The animals were then grouped by random selection. All animals were examined daily throughout the post-irradiation phase of the experiment and blood samples were taken on Day 1, 2, 3, 5, 8, 10, 15 and 29 following irradiation. The samples were taken by jugular puncture, using a 21-guage needle and evacuated tubes. Heparin was used as an anticoagulant. The leukocytes were diluted with a solution of 0.05 per cent methyl violet in 5 per cent acetic acid, for enumeration. For the determination of packed volume of erythrocytes, Wintrobe's hematocrit tubes were centrifuged at 2,700 revolutions per minute for 30 minutes. Hemoglobin concentrations were determined by the method recommended by Cannan (1).

RESULTS AND DISCUSSION

No clinical manifestations attributable to the whole-body exposure were observed during the post-exposure period. The animals remained alert, maintained their appetite, made normal weight gains, and appeared contented.

TABLE 1

Number of Animals and X-ray Exposure per Group

Group	Number of animals	X-ray dose
I	6	None
II	6	15 r
III	6	30 r

Exposure conditions: 250 kv, 30 ma
2.5 mm Cu and 1.0 mm Al filters
Distance to midline of animal: 183 cm
Dose rate: 318 r/minute in air
H. V. layer: 3.5 mm Cu
Effective energy in air: 145 kev

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The lambs which received 30 r of total-body x irradiation showed a significant decrease in lymphocytes when the three post-irradiation values were compared with the pre-treatment values (Figure 1). This decrease is reflected in total leukocyte levels (Figure 2). The decrease in lymphocytes in the animals that received 15 r is not considered significant under the conditions of the experiment.

No significant changes occurred in neutrophil, packed-cell volume, and hemoglobin levels (Table 2).

ACKNOWLEDGMENT

The technical assistance of M. E. Kerr and personnel of the Experimental Animal Farm and Biological Analyses Operations is gratefully acknowledged.

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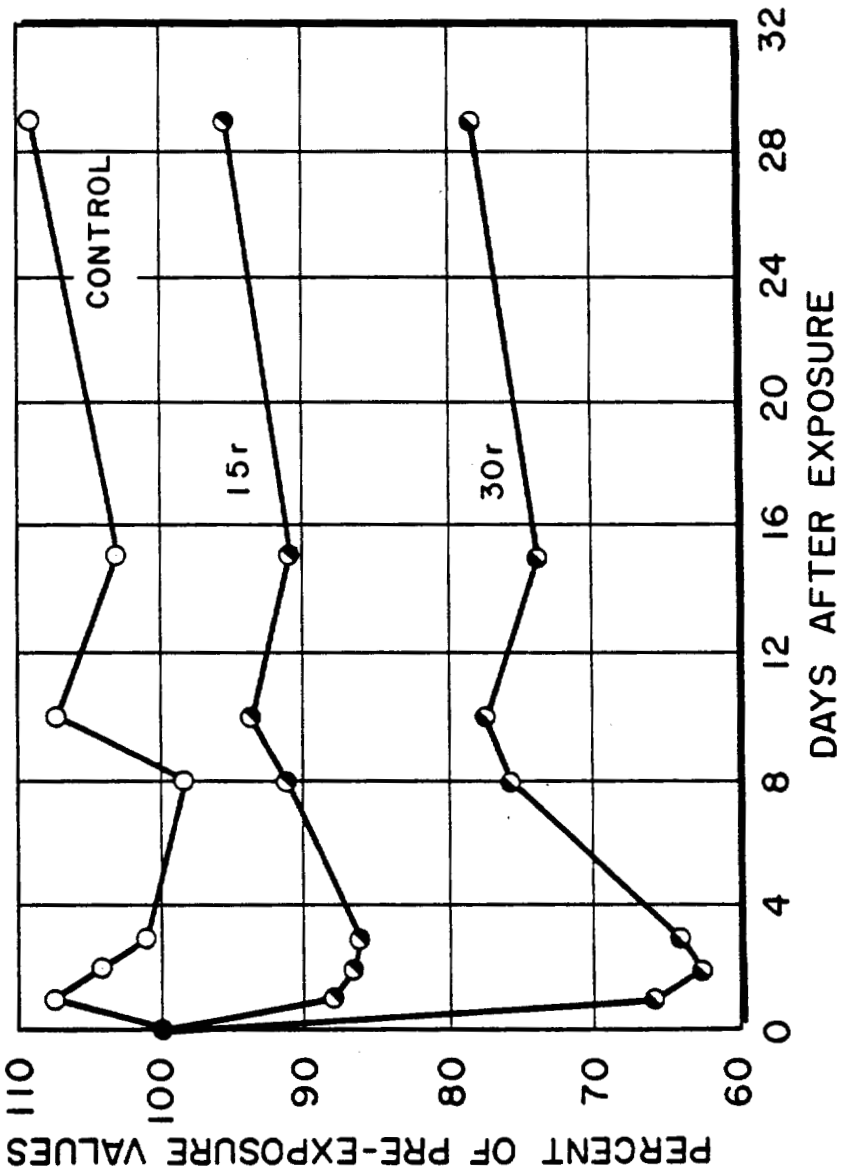

FIGURE 1

Per Cent of Pre-Exposure Lymphocyte Values in Control and Exposed Groups.

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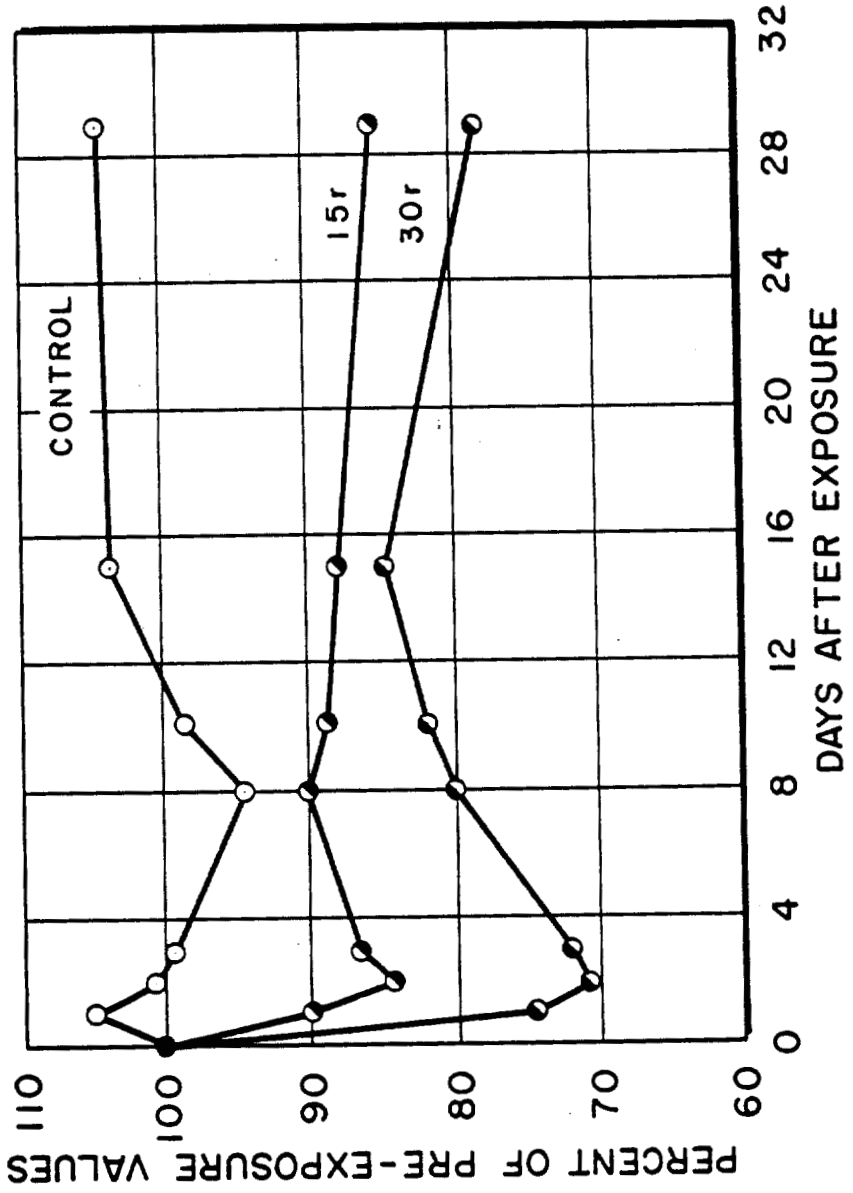

FIGURE 2
Per Cent of Pre-Exposure Leukocyte Values in Control and Exposed Groups.

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TABLE 2

Hematological Values

Constituent	Days after exposure	No. of samples	Control	15 r	30 r
Packed-cell volume	pre-treatment	18	37.4 ± 1.3*	40.1 ± 1.9*	40.2 ± 1.7*
	1	6	39.3 ± 1.4	42.8 ± 4.3	40.4 ± 3.2
	2	6	35.8 ± 2.6	38.0 ± 4.3	37.6 ± 3.0
	3	6	35.5 ± 3.5	36.5 ± 3.6	36.7 ± 4.4
	8	6	35.0 ± 3.8	36.3 ± 3.5	36.7 ± 3.5
	10	6	35.7 ± 3.5	38.9 ± 3.3	37.4 ± 2.5
	15	6	34.2 ± 2.1	35.6 ± 3.5	35.5 ± 2.7
	29	6	41.1 ± 6.0	41.5 ± 4.7	44.4 ± 4.6
Hemoglobin (g/100 ml)	pre-treatment	18	12.05 ± 0.20	12.62 ± 0.41	12.32 ± 0.44
	1	6	12.45 ± 0.45	13.18 ± 0.96	12.82 ± 0.66
	2	6	11.80 ± 0.63	12.41 ± 1.01	12.22 ± 0.69
	3	6	11.95 ± 0.48	12.05 ± 1.15	12.12 ± 1.05
	8	6	11.69 ± 0.79	12.12 ± 1.07	11.67 ± 0.66
	10	6	11.99 ± 0.90	13.01 ± 0.69	12.31 ± 0.73
	15	6	11.36 ± 0.54	11.72 ± 0.71	11.65 ± 0.73
	29	6	11.00 ± 0.48	11.46 ± 0.79	11.53 ± 1.03
Leukocytes (cells x $10^{-3}/mm^3$)	pre-treatment	18	9.58 ± 0.81	11.35 ± 0.81	12.76 ± 1.04
	1	6	10.08 ± 1.46	10.20 ± 2.20	9.51 ± 2.12
	2	6	9.67 ± 1.49	9.55 ± 2.27	8.97 ± 1.90
	3	6	9.53 ± 1.27	9.82 ± 1.67	9.14 ± 1.58
	8	6	9.03 ± 0.94	10.20 ± 2.12	10.08 ± 2.49
	10	6	9.44 ± 1.64	10.07 ± 2.32	10.44 ± 2.59
	15	6	9.95 ± 1.53	9.99 ± 2.27	10.82 ± 1.90
	29	6	10.02 ± 1.07	9.68 ± 1.22	9.99 ± 0.86
Neutrophils (cells x $10^{-3}/mm^3$)	pre-treatment	18	2.80 ± 0.29	2.98 ± 0.36	3.18 ± 0.51
	1	6	2.78 ± 0.43	3.13 ± 0.89	3.24 ± 0.99
	2	6	2.66 ± 0.74	2.63 ± 0.97	2.79 ± 1.18
	3	6	2.74 ± 0.43	2.85 ± 0.65	3.01 ± 0.81
	8	6	2.38 ± 0.35	2.89 ± 0.71	2.81 ± 1.25
	10	6	2.19 ± 0.40	2.57 ± 0.43	2.94 ± 1.05
	15	6	2.97 ± 1.26	2.73 ± 0.74	3.81 ± 1.78
	29	6	2.60 ± 0.29	2.70 ± 0.44	2.52 ± 1.09

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TABLE 2 (Contd.)

Constituent	Days after exposure	No. of samples	Control	15 r	30 r
Lymphocytes (cells x $10^{-3}/mm^3$	pre-treatment	18	6.61 ± 0.60	7.81 ± 0.60	9.33 ± 0.88
	1	6	7.11 ± 1.55	6.87 ± 1.47	6.15 ± 1.11
	2	6	6.89 ± 1.04	6.76 ± 1.63	5.85 ± 0.61
	3	6	6.67 ± 1.18	6.73 ± 1.91	5.98 ± 1.01
	8	6	6.50 ± 0.79	7.12 ± 2.04	7.07 ± 1.59
	10	6	7.11 ± 1.51	7.31 ± 1.99	7.32 ± 1.58
	15	6	6.82 ± 1.16	7.10 ± 1.84	6.87 ± 1.44
	29	6	7.24 ± 1.13	7.47 ± 1.27	7.34 ± 0.77

*95% confidence intervals about the mean

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1086668

MINIATURE PIG METABOLISM CAGE FOR RADIOBIOLOGICAL STUDIES

V. G. Horstman, L. A. George, Jr.*, M. E. Kerr
and L. K. Bustad

A glove-box type metabolism cage for the effective separation of urine and feces from various sizes of male miniature pigs administered high levels of alpha- and beta-emitting isotopes is described.

It was necessary to fabricate a metabolism cage to safely accommodate pigs of various sizes and to effect a separation of urine and feces before undertaking certain metabolism studies in miniature pigs involving administration of high-level doses of alpha- or beta-emitting isotopes. The cage was constructed of stainless steel frame with plexiglass (Rohm and Haas Company) sides for easy observation of the pig. Three eight-inch glove ports on each side were fitted with either rubber gloves or plastic bags for access to the pig. Glove ports were provided in the lower compartment for collecting samples. The cage is completely air tight and has its own air filtering system.

Urine and feces, collected in quart jars by separate funnels (Figure 1), are effectively separated by the proper adjustment of the cage to fit each pig. Adjustment is accomplished by use of easily installed floor plates and by movable baffle plates (Figure 2) which change the inside dimensions of the cage. Movement of the pig can be restricted by adjustments prior to the start of the experiment to insure good separation of urine and feces.

The excreta are collected in wide-mouthed quart jars that can be removed in plastic bags and sealed, preventing spread of contamination. The pig can be fed and watered from outside the cage by means of a pipe leading to a trough embedded in the floor of the cage. The pig seems to adapt well to this type of confinement.

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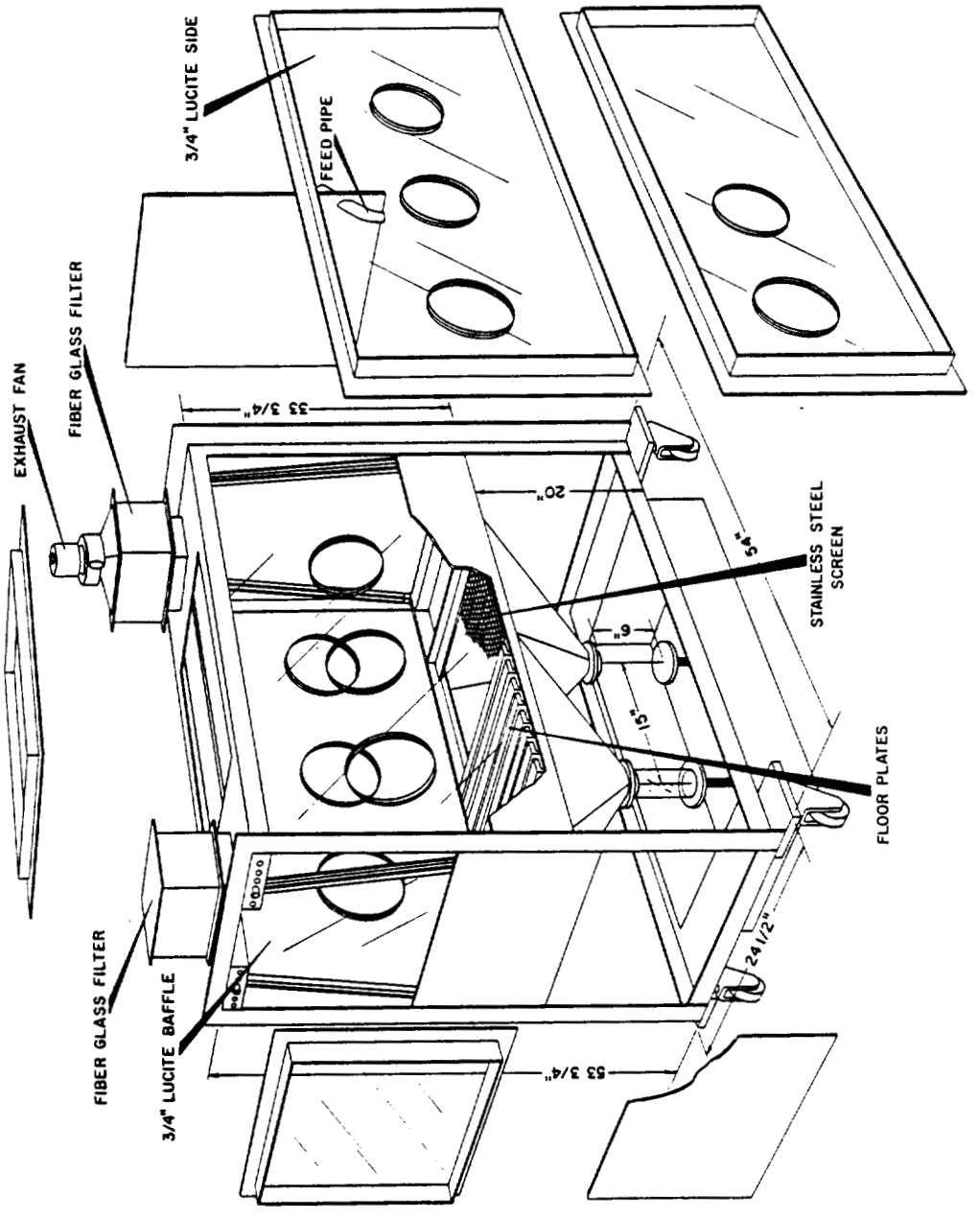

FIGURE 1
Side View Drawing of Metabolism Cage.
Note Removable Floor Plates for Length Adjustment, Feeding Facilities, and Collection Jars in Place.

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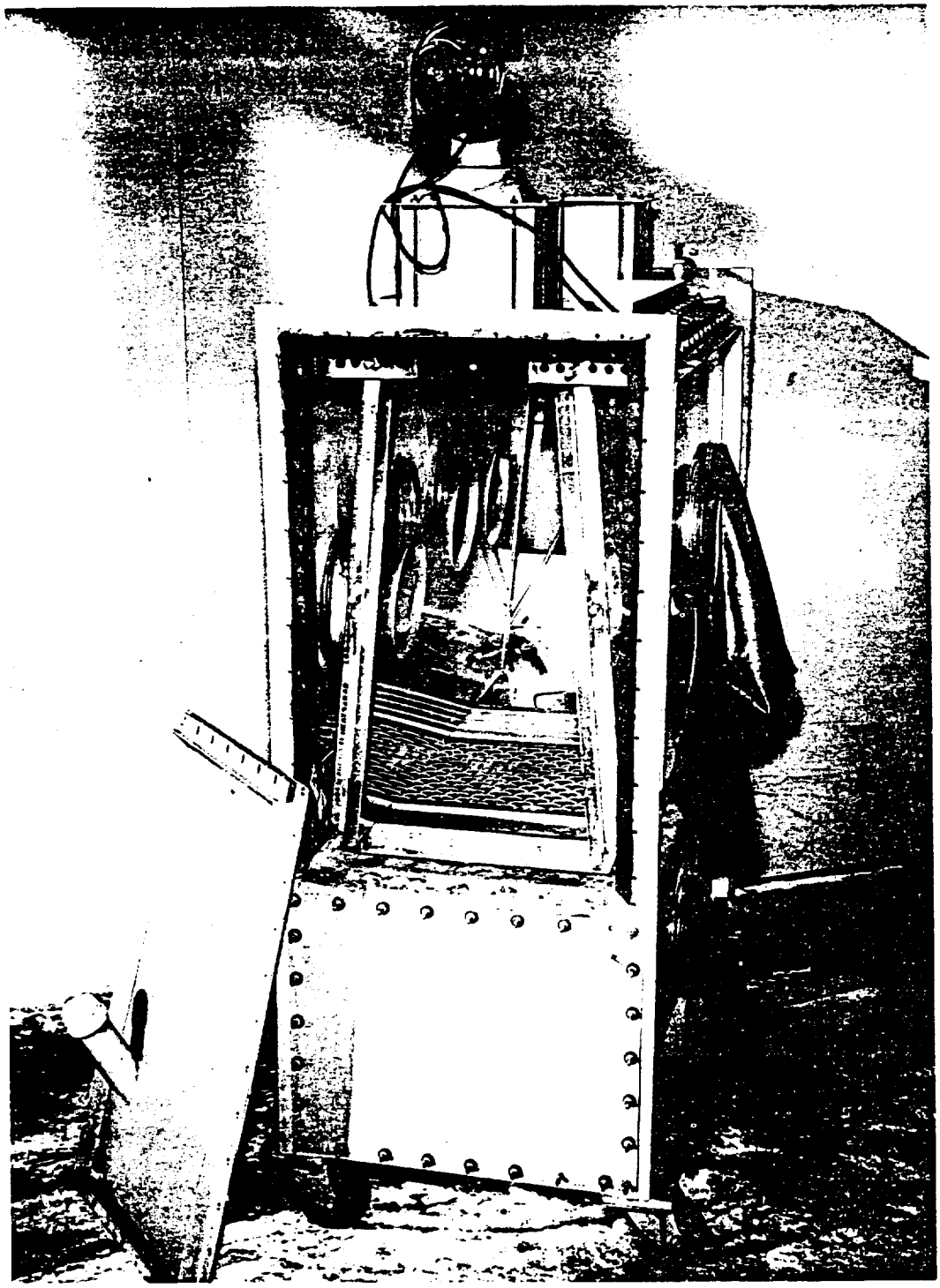

FIGURE 2

Front View Photograph of Metabolism Cage.
Note Baffle Plates for Adjusting Width

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This cage has been used successfully with alpha-emitting isotopes. Decontamination of the cage is simplified by spraying it with a strippable paint, polyvinyl butyral dispersion, No. D-1000*, before each metabolism study.

In the future it is planned to utilize the cage for experiments involving the use of beta-emitting isotopes.

ACKNOWLEDGMENT

Grateful acknowledgment is made for the assistance of W. D. Martin, Mechanical Design Operation.

*Manufactured by Shawinigan Resins Corporation, Springfield, Mass.

1086672

GROSS EFFECTS OF BETA RAYS ON THE SKIN

L. A. George, Jr.* and L. K. Bustad

Observations were made over a two-hundred day period on the comparison of the gross lesions in the skin of sheep, swine, and rabbits, each exposed to a single dose of beta irradiation from the application of Sr^{90} or P^{32} plaques. At 2,000 rad, no appreciable species difference was noted, except for a slight pigmentation in the sheep; 8,000 rad increased the intensity of pigmentation in the sheep, stopped hair regrowth in the swine, and resulted in incomplete epithelial repair in the rabbit. At 16,000 rad no species evidenced hair regrowth.

Several papers dealing with the effects of beta irradiation of skin have appeared in the literature during the past few years (1, 2, 3), but generally they have emphasized the histological nature of the lesions. The object of the study reported in this paper was to determine the amount of variation in the skin reaction of different species of domestic animals after they were exposed to the same dose of beta irradiation, emphasizing gross lesions rather than histological lesions.

METHODS

Animals used in this experiment consisted of two Palouse white pigs, two Suffolk sheep, and six New Zealand white rabbits. The pigs and rabbits were two and three months of age, respectively, and the sheep were eight months of age at the time of beta ray exposure. All of the animals were housed in the same laboratory room, but in separate pens before and after exposure. One closely clipped, thoroughly washed area on each side of the rabbits, and three areas on each side of the sheep and pigs were selected for irradiation.

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All of the animals were anesthetized during exposure with Dial in Urea-Urethane (Ciba), a long-duration anesthetic.

The beta sources used in the experiment were a P^{32} plaque, manufactured by Oak Ridge National Laboratories, having a surface dose rate of 5,000 rad/hour, and a locally fabricated Sr^{90} source with a surface dose rate of 8,700 rad/hour.

The exposure levels selected for study were 2,000, 8,000, and 16,000 rad. The P^{32} source was used for the (beta radiation) exposure on half of the animals and the Sr^{90} source for the other half.

RESULTS AND DISCUSSION

There was considerable difference in the reaction of the skin of the three species of animals studied when exposed to the same dose level of beta irradiation, especially at the two highest dose levels. The gross appearance of the lesions at each dose level and at various times after exposure is shown in Tables 1, 2, and 3. Due to the small number of animals used in the experiment, and to individual variation in the skin reaction after exposure to the same beta ray source (4), no attempt is made to discuss differences in skin reaction caused by the two different sources.

Rabbit skin is more sensitive to beta irradiation than sheep or swine skin. Complete epithelial repair occurred in sheep and swine at all levels of irradiation studied, while the rabbit evidenced incomplete epithelial repair from 8,000 rad or more. At 8,000 rad, swine hair follicles showed much more sensitivity to beta irradiation than did sheep or rabbit. There was no hair regrowth in swine, but sparse regrowth of sheep and rabbit hair.

The pigmentation observed in the sheep skin (Figure 1) was not evident in the swine or rabbit. Possibly this variation would not be so apparent if different breeds of swine and rabbits had been used in this experiment. Various colored breeds would probably represent a rather wide degree of sensitivity to certain effects (erythema and pigmentation, particularly).

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TABLE 3

16,000 Rad of P^{32} or Sr^{90} Beta

Days	Rabbit	Sheep	Swine
0 - 24 hrs.	Slight erythema.	Slight erythema and marked swelling.	Intense erythema.
1 - 8	Erythema.	Swelling decreased and skin dry, bluish-pink, denuded and sensitive to touch.	Very slight to no erythema.
8 - 18	Desquamation after 18th day.	Skin grayish-blue, dry, non-resilient, but less sensitive to touch. Epidermis showing dry necrosis.	Re-appearance of erythema after 10th day. Maximum redness on 15th to 17th day, followed by necrosis of epidermis.
18 - 28	Epidermis necrotic 20th day. Became detached from underlying tissue on 28th day, leaving moist exuding lesion.	Continued dry necrosis with development of thick, stiff, non-viable epithelial disc.	Complete loss of necrotic epidermis, revealing bright red, moist lesion. Scab formed on surface.
28 - 45	Scabs formed, then dislodged, leaving moist exuding lesions of successively smaller diameter.	Dry necrotic epidermis flaking off after 36th day, revealing intensely pigmented peripheral zone and non-pigmented central zone.	Continued repair beneath debris-contaminated scabs. Regenerating epidermis growing in from periphery.
45 - 60	Epithelization inward.	Necrotic epidermis flaking off, revealing larger areas of smooth non-pigmented regenerated epithelium in center.	Repair process continued.
60 - 100	Epithelization stopped after 60th day. Central irregular area covered by scab, often dislodged.	Epithelization complete by 60th day. Epidermis smooth, pink, and non-pigmented, surrounded by zone of intense pigmentation.	Exposed areas completely healed by smooth, dry, scaly epidermis by 15th week. No regrowth of hair.
100 - 200	Two of four areas completely healed by 15th week but breaks in regenerated epithelium left moist, exuding lesions through 30th wk.	No change through the 30th week.	No change through the 30th week.

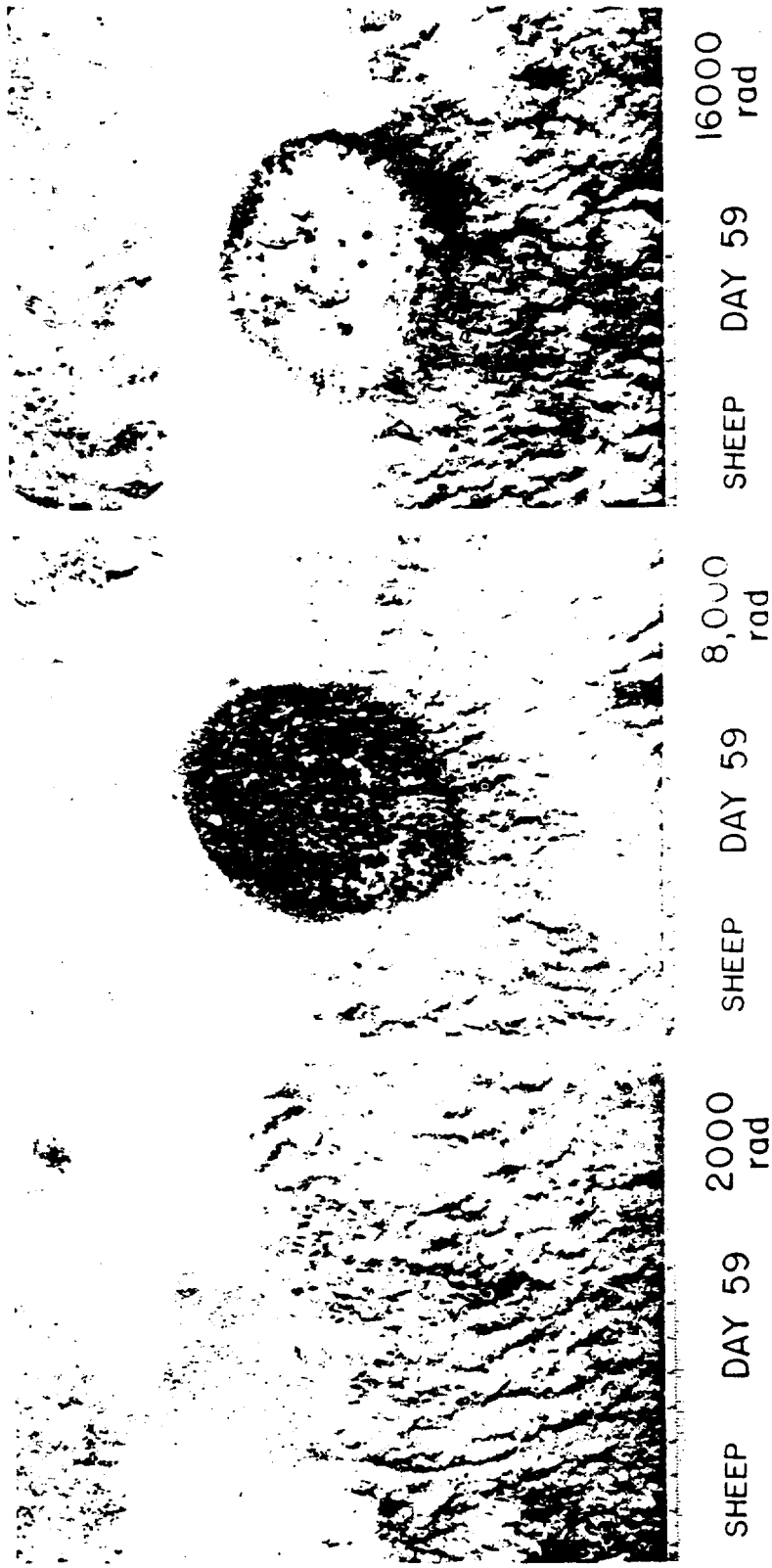

FIGURE 1
The Relationship of Dose to Pigment Production in Sheep Skin.

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ACKNOWLEDGMENTS

The technical assistance of Dr. E. E. Donaldson of the Physics Research Operation and M. E. Kerr and members of the Experimental Animal Farm staff is gratefully acknowledged.

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1086677

ABSORPTION AND DECONTAMINATION OF PLUTONIUM ON RATS

J. E. Ballou and W. D. Oakley*

Absorption through rat skin of plutonium from a solution containing PuF_4 in nitric acid was comparable to that previously measured for other nitric acid solutions of plutonium. The absorption of TBP-complexed plutonium in carbon tetrachloride solution was approximately four times greater. The ability to remove plutonium from pieces of excised pig skin was found to be a suitable preliminary test of the efficiency of decontaminating agents.

Previous studies on the percutaneous absorption of plutonium and its removal from rat skin have been presented in other reports (1). Further experiments reported here include the determination of the percutaneous absorption and decontamination of other solutions and the development of a procedure for screening decontaminating agents.

METHODS

Young adult female rats were exposed to the plutonium solutions by methods and procedures described in a previous report (1). The pig skin employed in decontamination studies was obtained at autopsy, clipped free of hair and frozen until thawed for use. Plutonium analysis on selected tissues was performed by standard methods (2).

RESULTS AND DISCUSSION

Absorption of Process Solutions

The absorption of two plutonium solutions at several time intervals after exposure is shown in Table 1. The PuF_4 was applied to the skin as a solution in nitric acid. The TBP complexed plutonium was in solution of

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tributyl phosphate and carbon tetrachloride with traces of nitric acid. Results indicate that the PuF_4 solution is of no more serious hazard concern than other nitric acid solutions of plutonium of the same mortality. The TBP complexed plutonium was absorbed to a significantly greater extent by the rat but was effectively removed by standard decontaminating procedures.

TABLE 1

Percutaneous Absorption of Plutonium
As Affected by Length of Exposure

Length of exposure	PuF_4 average % applied dose absorbed*	TBP-complex average % applied dose absorbed
15 min.	0.004	0.04**
1 hr.	0.003	
5 hr.	0.02	
24 hr.	0.1	0.4
5 d.	0.2	
6 d.		0.7

* Average of 5 rats or more.

** Animals were decontaminated by Standard Hanford Method after 15 minutes exposure. They were subsequently killed 5 days after exposure and decontamination.

Screening of Decontaminating Agents

The ability to remove plutonium contamination from small pieces of pig skin was found to be a rapid and reproducible preliminary test of the efficiency of decontaminating agents. The degree of reproducibility is indicated by the results shown in Table 2.

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REMOVAL OF PLUTONIUM FROM PIG SKIN

L. A. George, Jr.*, N. L. Dockum, and L. K. Bustad

Several methods of decontaminating plutonium-contaminated skin were studied. After contaminating localized areas of pig skin, different decontamination methods were tested. Results indicate the most effective of the decontamination methods tested involved application to the skin of a plastic adherent and its later removal by peeling.

This report describes a pilot study performed on swine for the purpose of testing the effectiveness of different methods of skin decontamination.

METHODS

Two young adult swine weighing approximately 80 kg were washed with soap and water, clipped with electric clippers, and placed in glove-box type cages one week before being contaminated. This measure made it possible to determine which regions on the sides or back of the animal would be completely free of abrasions resulting from movement within the confining cage. It was found that an area approximately 25 cm wide and extending along the back from the shoulders to the hips was completely free of damage.

The animals were removed from the cages and anesthetized with intravenously administered nembutal during the contamination and decontamination procedures. The induction dose of nembutal was 2 mg per kg, and the animals were maintained under anesthesia for five hours by repeated intravenous doses.

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**Penobarbital sodium (Abbott)

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The contaminant used in this experiment was plutonium dissolved in a mixture of carbon tetrachloride, tributyl phosphate, and nitric acid. The concentration of plutonium was 37 mg/ml.

Several areas on the dorsolateral surface of the pig, each of which measured approximately one square centimeter, were contaminated by applying one drop of the plutonium solution on the skin and gently spreading it over the desired area. A period of 10 to 15 minutes was allowed to elapse between the time the contaminant was applied and decontamination procedures were begun. Table 1 lists the chemical agents tested and briefly describes the manner in which they were used. Because it was desired to determine whether the decontamination agents being tested influenced the absorption of the contaminant, it was decided to base the evaluation of the effectiveness of the different methods on the relative concentrations of alpha activity on the surface of the skin, as observed in autoradiograms.

In one animal the biopsies were removed 18 hours after the contamination and decontamination procedures were completed. In the other animal 16 days elapsed between the decontamination procedures and the removal of the biopsies. Skin biopsies were fixed in acetone and in formalin. Autoradiograms and hemotoxylin-and-eosin-stained sections were prepared from tissue fixed by each of these methods (1, 2).

RESULTS AND DISCUSSION

Table 1 summarizes the results obtained with the different methods of decontamination. The efficacy of these methods depends upon the way in which the contaminant is deposited and adheres to the surface of the skin. Examination of the autoradiograms and stained sections shows that the contaminant deposits as a precipitate of small particles ranging in size up to 2 microns* in diameter (Figures 1 and 2). These particles appear to

*Measured under oil immersion with a calibrated stage and ocular micrometer.

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TABLE 1

Concentration of Alpha Activity Determined by Autoradiograms

Decontamination method	Concentration	
	Epithelial surface	Along hair shaft
Washed with soap and water, dried, applied Aeroplast. Removed Aeroplast one hour later.	Slight	None
Washed with soap and water, dried, applied cuticle remover, washed again, dried, and applied Aeroderm. Aeroderm removed one hour later.	Slight	Trace
Washed with soap and water, dried, applied cuticle remover, washed again, and dried.	Heavy	Slight
Washed with soap and water, dried, applied acetone, and washed again.	Heavy	Slight
Washed with soap and water, dried, applied $KMnO_4$ three times and removed with $NaHSO_3$, then washed and dried again.	Moderate	Slight
Washed with soap and water, dried, tributylphosphate solution applied, and then washed again.	Moderate	Slight
Washed with soap and water only and dried	Heavy	Slight
* A solution of Zr citrate and Ca EDTA** in combination, then washed and dried.	Moderate to Slight	None
* Ca EDTA, then washed, and dried.	Moderate	None
* Zr citrate, then washed and dried.	Moderate	Slight
* $KMnO_4$ and $NaHSO_3$ technique, then washed and dried.	Moderate	Slight

*This method did not include washing with soap and water initially, but treatment only with the material indicated, and then washed and dried.

**Calcium ethylenediaminetetracetate.

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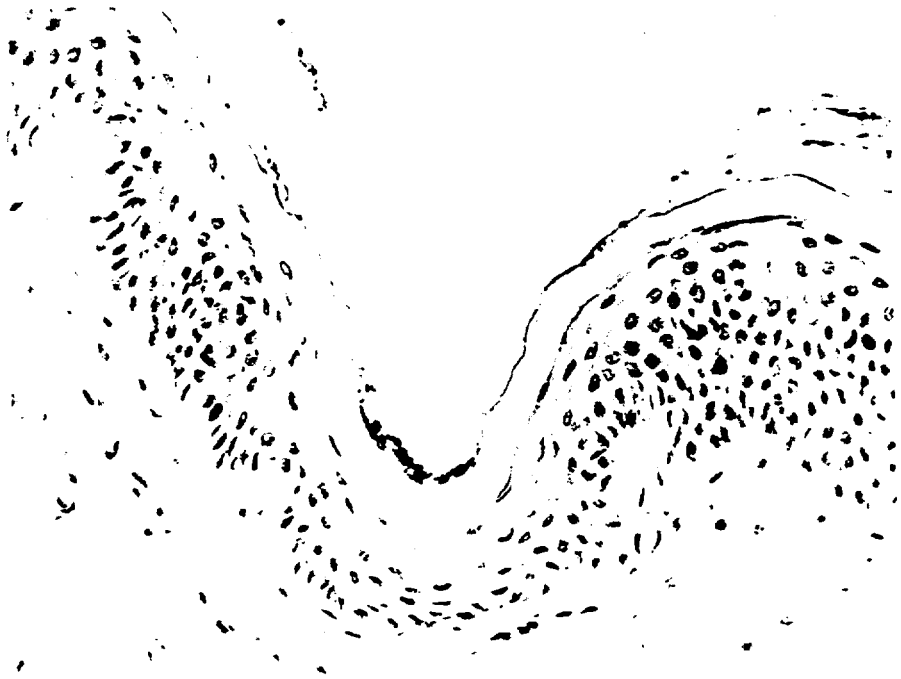

FIGURE 1

Microscopic Section of Pig Skin. Showing the Deposition of Plutonium-Containing Material in an Epithelial Groove. (H and E Stain, 300X)

FIGURE 2

Unstained Autoradiogram from a Section Adjacent to Figure 1. Showing Alpha Tracks from the Deposited Material. (300X)

AEC-GE RICHLAND, WASH.

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adhere tightly to the outer surface of the cornified layer of the stratum corneum and often concentrate in the irregularities of this layer.

The results indicate that the most effective of the decontamination methods tested involved the application of a plastic adherent material to the skin and its later removal by peeling. The adherent tested in this case was Aeroplast*, a liquid surgical dressing used for spraying a protective covering over minor wounds and burns.

This method of decontamination amounts essentially to mechanical removal of the contaminant. This is accomplished by virtue of the apparent firm bond developed between the plastic spray and the contaminated surface layer of stratum corneum which is removed when the plastic material is peeled from the skin. Figure 3 shows the thickness of the stratum corneum of skin removed from the volar surface of a human finger. It would appear that several layers of this cornified portion of the skin could be removed over a period of several days or weeks by successive applications of the plastic adherent, without harm to the deeper layers of the skin. One application of the adherent was applied in the case of the pig skin, but it is apparent (Figures 4 and 5) that an appreciable thickness of the stratum corneum still remains and could possible be removed by repeated applications of the adherent. These findings are in general agreement with those of Wilson in which he mentioned the use of Elmer's glue (a water-soluble casein base glue) as an adherent for this method of decontamination (3).

There was no appreciable difference in the over-all effectiveness of the other decontamination methods tested. Variable migration of the contaminant down the side of the hair shaft was observed in most of the sections regardless of the method of decontamination used (Figures 6 and 7).

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*"Aeroplast" - manufactured by Aeroplast Corporation, Dayton 3, Ohio.

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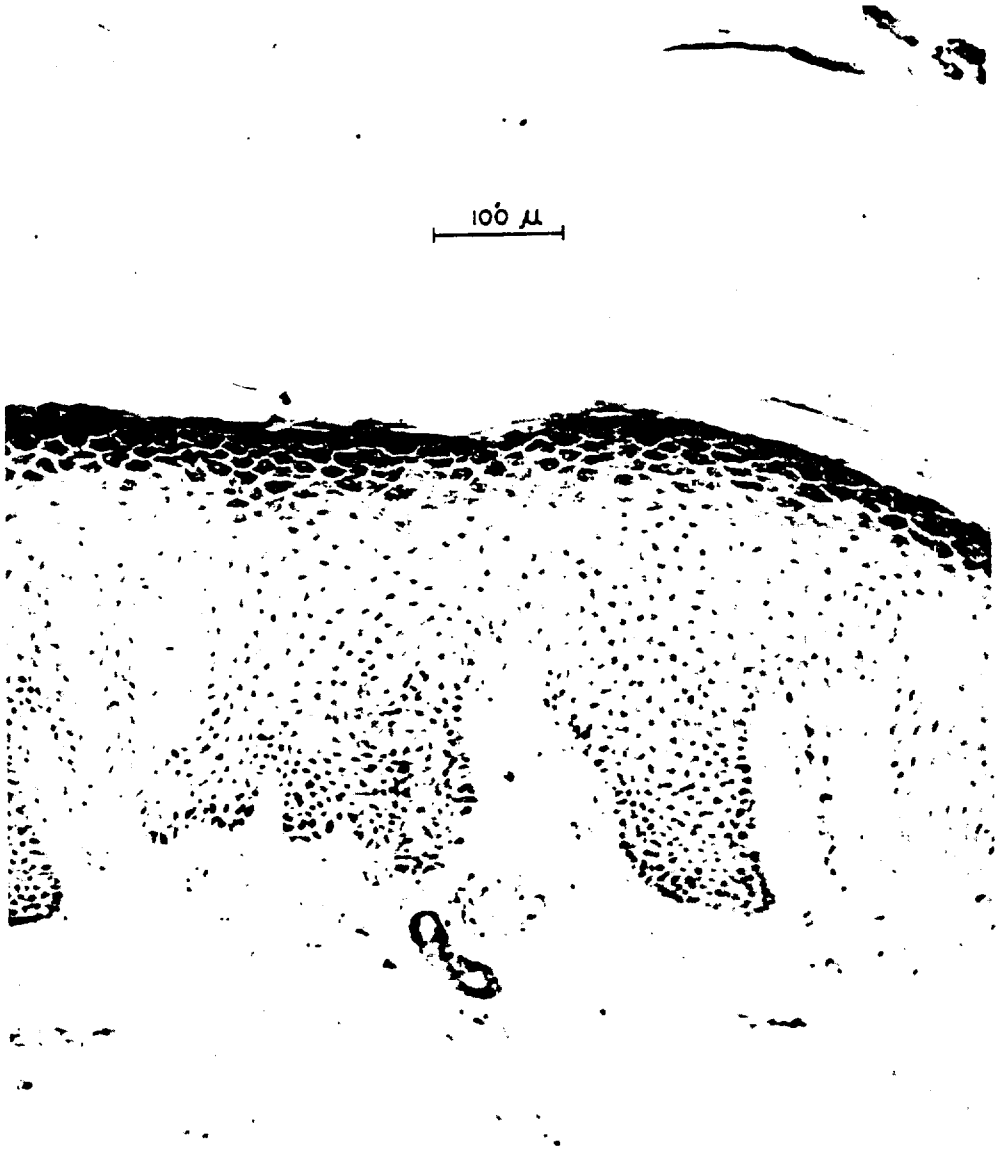

FIGURE 3

Stained Section of Human Skin from the Volar Surface of the Finger Showing the Thickness of the Stratum Corneum. (H and E stain. 130 X)

AEC-GE RICHLAND, WASH.

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FIGURE 4

Microscopic Section of Pig Skin Following Decontamination with One Application of Aeroplast. Note that a Considerable Amount of Stratum Corneum Remains Intact. (300 X).

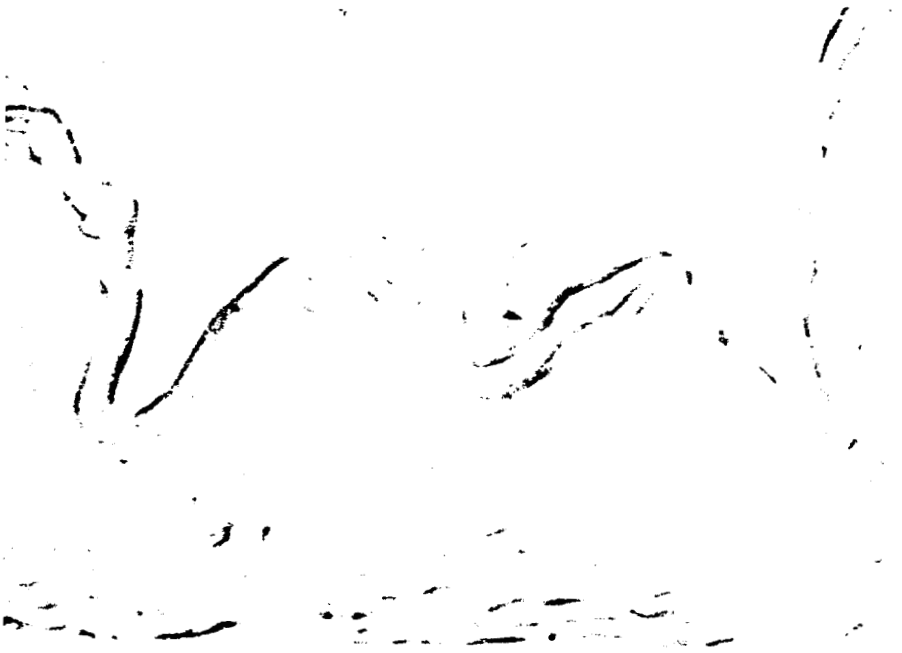

FIGURE 5

Phase Photomicrograph of Unstained Autoradiogram from the Same Area as Figure 4 Showing Evidence of Residual Contamination on the Epidermal Surface. (300 X)

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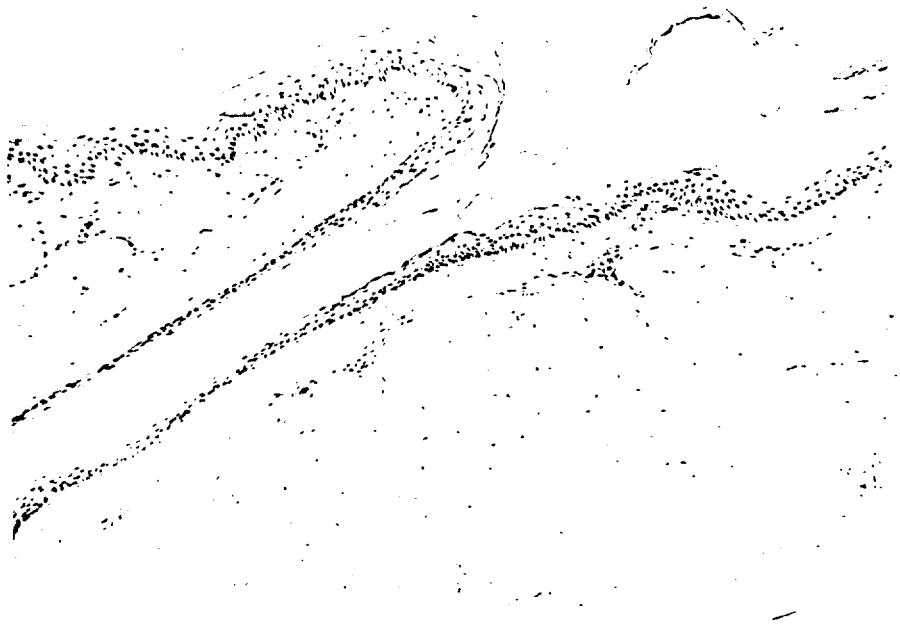

FIGURE 6

Microscopic Section of Pig Skin Showing Portion of a Hair Follicle. (H and E stain. 65 X)

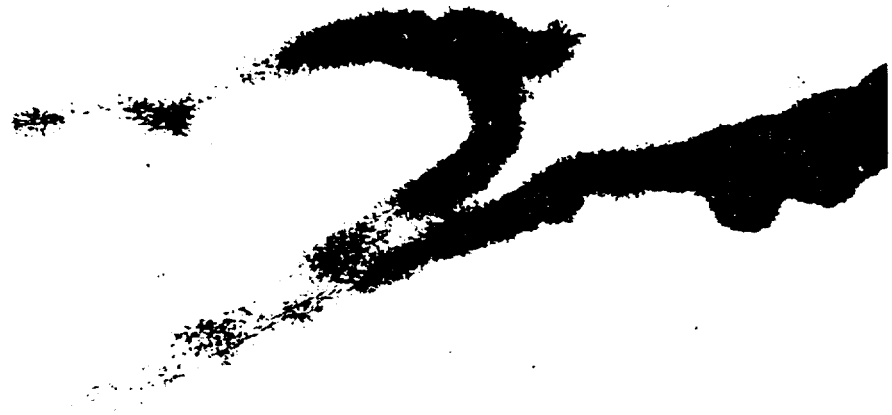

FIGURE 7

Unstained Autoradiogram of Decontaminated Pig Skin from the Same Region as Figure 6, Showing Evidence of Residual Plutonium on the Epidermal Surface and in the Superficial Portion of the Hair-Follicle. (65 X)

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The autoradiograms of skin biopsies removed from the second animal 16 days after the contamination and decontamination procedures showed heavy contamination of the epidermal surface, especially in the irregularities of this surface, and definite migration of the contaminant down the side of the hair shafts. One of the biopsy sections from this second animal showed selective deposition of the contaminant in a sebaceous gland (Figures 8 and 9). It is presumed that this deposition occurred in conjunction with movement of the contaminant down the side of the hair shaft, since the excretory duct of a sebaceous gland opens into the neck of a hair follicle.

These results are based upon microscopic examination of the acetone-fixed and prepared tissue sections only. Examination of the formalin-fixed tissue sections revealed that formalin exhibited an apparent leaching effect upon the contaminant. The formalin-fixed sections, which were directly adjacent to the acetone-fixed sections and thus received identical treatment, uniformly showed less dense activity than the acetone-fixed sections. These findings are in agreement with the observations of other workers (4, 5).

ACKNOWLEDGMENTS

The assistance of S. Marks, Consultant in Pathology to Hanford Laboratories; J. E. Ballou, Metabolism Operation; Radiological Monitoring Operation personnel; V. G. Horstman, M. E. Kerr, and the staff of the Experimental Animal Farm Operation is gratefully acknowledged.

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1086689

PREPARATION OF AQUEOUS SUSPENSIONS OF $Ru^{106}O_2$ AND $Pu^{239}O_2$

V. H. Smith and R. W. Wager

The preparation of aqueous suspensions of radioactive ruthenium oxide and plutonium oxide particles is described.

Animal experiments required suspensions of radioruthenium oxide and plutonium oxide for intratracheal injection (1, 2). Suspensions of plutonium were previously prepared by grinding the oxide to a powder and classified as to particle size (3). However, this method was not easily applicable to Ru^{106} because the grinding and classification was a long, tedious process and the radiation from this strong beta-gamma emitter limited the amount of material that could be handled. In addition, the grinding process required large amounts of carrier. A method was therefore developed to produce a suspension of particles in the desired size range and of minimum carrier, and of decreased radiation hazard.

METHODS

Radioruthenium trichloride was converted to the mixed, hydrated oxide with NaOH, oxidezed to the quadrivalent state with H_2O_2 and dried at 150 C to yield RuO_2 particles. The particles were deaggregated by extended ultrasonic agitation and dialyzed against ion-exchange resins to remove soluble ions. The final suspension was made using an aqueous solution of Pluronics* as a suspending agent.

Plutonium oxide particles were obtained as a powder, dialyzed and suspended in aqueous Pluronics.

* Wyandotte Chemicals Corporation, Wyandotte, Michigan.

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RESULTS AND DISCUSSION

Ruthenium oxide particles so prepared usually ranged in size from 0.5 to 2.5 microns diameter with 90 per cent in the 1 to 2 micron range. A typical 5 ml suspension, before dilution, had a concentration of 5.4 mc/ml. The particle size could be varied by altering the initial conditions of precipitation or by "aging" the suspension. In general, there was a tendency to produce particles of colloidal proportions which decreased the yield of larger particles. Dialysis removed less than 0.01 per cent of the original material and it is doubtful that this procedure was justified in view of the losses entailed in removing the particles from the dialysis sack. Particles over 1.5 microns were usually aggregates.

The plutonium oxide powder was obtained locally* and ranged in diameter from 0.5 to 3 microns with about 80 per cent of the particles 0.5 to 1.5 microns.

Tween-80**, polyoxyethylene sorbitan monooleate, was used as a suspending agent in earlier work (3), and had shown an antitumorigenic effect which was undesirable. A new suspending agent was formulated using Pluronics. These non-ionic, polypropyleneglycol-ethylene oxide polymers appeared to have no effect on tumor production (1).

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*Kindly provided by H. H. Hopkins, Jr., and R. C. Smith, Research and Engineering Operation, Chemical Processing Department.

** Atlas Powder Company, Wilmington, Delaware.

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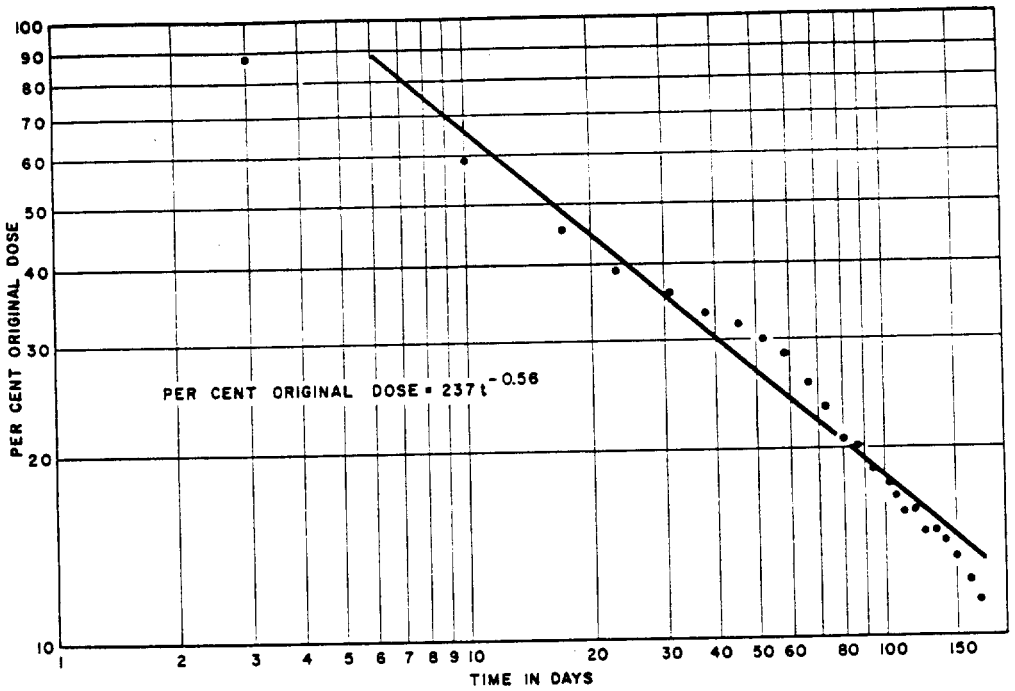

FIGURE 1

Whole Body Turnover of Ru^{106}

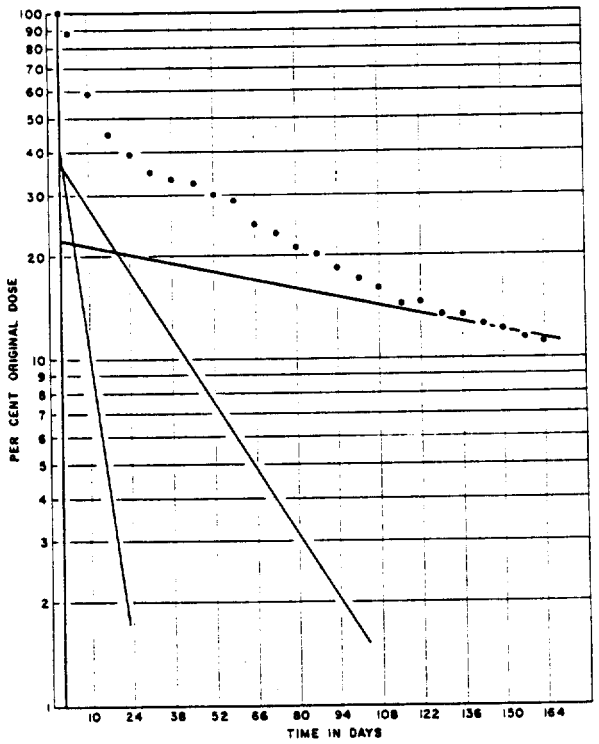

FIGURE 2

Whole Body Turnover of Ru^{106}

AEC-GE RICHLAND, WASH.

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determine the curve best fitting the data. The equation for the curve is a power function of time, per cent of the original body dose equals $237t^{-0.56}$ (from 10 to 164 days).

When the data are plotted on semi-logarithmic scale in Figure 2, the resulting curve may be resolved into three components to represent the period covered - 164 days. Thirty-nine per cent of the original dose was lost with a half-time of 4.3 days. This relatively rapid turnover was probably due to ciliary action in the bronchi, sweeping the particles out of the respiratory passages. Thirty-eight per cent was removed with a half-time of 24 days and 24 per cent at a much slower rate with a half-time of 164 days. The latter was not unexpected considering the insoluble nature of ruthenium oxide. Twelve per cent of the administered dose was retained after 164 days.

The distribution of the remaining Ru^{106} in the tissues of the mice is shown in Table 1 and is expressed as the per cent of the total body burden at the time of sacrifice (164 days). Mean values are given with 95 per cent confidence limits. The concentration of Ru^{106} in the tissues is also shown and is expressed as μc/gram wet weight of tissue. The lungs contained 92 per cent fo the total body burden, the remainder being almost entirely distributed throughout the bones, muscle, and skin. However, other than the lungs the tissues showing the greatest concentration of Ru^{106} were the lymph nodes, spleen, and ovaries. The high concentration in the lymph and spleen may be indicative of the mode of transfer from the lungs. The reason for a similarly high concentration in the ovaries is not immediately apparent but this observation may be of considerable importance in considering the genetic aspects of the gonad dose. *

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*The paper by Ballou and Thompson on "Distribution and Retention of Ruthenium" (this report) further suggests the importance of the gonads when considering the hazards of radioruthenium.

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TABLE 1

Tissue Distribution of Ru^{106}

Tissue	Per cent body burden			c/gram
	Mean values	95 per cent confidence limits		
Lungs	92.1	89.4	94.9	0.62
Bones	5.1	3.8	6.3	.0049
Muscle	2.9	2.2	3.6	.0017
Skin	2.8	1.8	3.8	.002
Lymph	1.7	1.3	2.2	.0454
Liver	1.6	1.3	1.8	.0039
Spleen	0.5	0.4	0.6	.0339
Kidney	0.2	0.2	0.3	.0022
Intestine	0.2	0.1	0.3	-
Stomach	0.08	0.06	0.1	-
Ovaries	0.01	0.01	0.02	.013
Adrenals	0.009	0.006	0.01	.003

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This demonstrated low rate of turnover from the lungs suggests that the origin of pathological changes following the inhalation of small quantities of insoluble radioactive particulates may be confined to the pulmonary tissues.

ACKNOWLEDGMENTS

Statistical analysis of the data by P. E. Leaverton, Experimental Statistics, Operation Research and Synthesis, is acknowledged.

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LUNG TUMORS FOLLOWING DEPOSITION OF PARTICULATE MATERIAL

L. A. Temple, D. H. Willard, V. H. Smith, and R. W. Wager

Insoluble, radioactive particles and a chemical carcinogen introduced into the lungs of mice increased the incidence of lung tumor at 180 days. Although a non-specific pneumonitis was seen after methylcholanthrene, $Ru^{106}O_2$, and $Pu^{239}O_2$ administrations, it was greater after methylcholanthrene and $Ru^{106}O_2$.

The per cent of mice with pulmonary papillary cystadenoma was used as an indicator to determine the possible carcinogenic nature of radioactive particulate material deposited in the lungs. A base line was established by treating different groups of mice with external radiation, a chemical carcinogen, and by comparing the results of a relatively innocuous treatment to intratracheally injected insoluble, radioactive, particulate material.

METHODS

One group of mice was exposed daily (5 days per week) to 10 r of x rays from a General Electric Maxitron operated at 250 KVP and 5 ma, added filtration from a Thoraeus III* filter. Dose rate was 1 r/minute and accumulated dose was 1,225 r.

Preparation of the insoluble, radioactive, particulate material used in this experiment is described in a previous paper of this report (1).

The non-radioactive particles were ground in an agate mortar and classified by sedimentation to the desired diameter (about 1 micron). An

*0.6 Sn + 0.25 mm Cu + 1 mm Al.

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aqueous mixture of 0.09 per cent "Pluronics"* F-68 and 0.01 per cent Pluronics L-61 was used as the suspending agent in all cases. A total volume of one-tenth milliliter was injected into each mouse as previously described (2).

Numbers of peripheral tumors were determined in excised lungs inflated with 80 per cent ethanol. The induced tumors are pearly white in color and situated close to the pleura so that most of them may be seen on gross inspection as shown in Figure 1. Tumors reported were confirmed by histological examination (3).

RESULTS AND DISCUSSIONS

Tumor incidence, as noted by the per cent of mice with tumor, 180 days after I. T. administration of insoluble, particulate material in BAF_1 mice, is summarized in Table 1. Using Pluronics as the suspending agent, an increase in tumor incidence was noted after lung deposition of chemical carcinogen, non-radioactive ruthenium oxide, $Ru^{106}O_2$, and $Pu^{239}O_2$. An increase in tumor incidence was also observed after whole-body x-irradiation. In an earlier experiment, when "Tween-80"** was used as the suspending agent, no increase in tumor incidence was observed except after lung deposition of a chemical carcinogen.

No synergistic or additive effects of chemical carcinogen and radioactivity as tumorigenic agents were observed, but $Ru^{106}O_2$ plus a chemical carcinogen are additive in forming a non-specific pneumonitis.

A partial sterilization of pretumorigenic cells by radiation is suggested by the lower tumor incidence noted after injection of dibenz [a, h] anthracene plus radioactive particulate material. On the basis of amounts

*Wyandotte Chemical Corporation
**Atlas Powder Company

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FIGURE 1
Peripheral Pulmonary Tumor

TABLE 1

Tumors* in Lungs of BAF_1 Mice After I. T. Administration of Particulate Material

Treatment	Amount of material injected	No. of mice	% mice with tumor	Average tumors/ animal	% mice with lung path**
None	---	138	17.0	.17	0.0
Methylcholanthrene	0.05 mg	16	69.0	1.8	19.0
Pluronics	0.1 mg	47	19.0	.21	0.0
Ruthenium Oxide (non-radioactive)	0.01 mg	46	28.0	.41	6.5
Total body x ray	1225 r	42	62.0	1.0	0.0
Total body x ray and methylcholanthrene	1225 r 0.05 mg	45	56.0	1.1	4.4
$Ru^{106}O_2$	3.0 μc	41	56.0	.78	42.0
$Ru^{106}O_2$	0.45 μc	44	23.0	.43	6.8
$Ru^{106}O_2$ and dibenz[a,h]anthracene	3.0 μc .05 mg	26	50.0	1.2	58.0
$Pu^{239}O_2$	.0033 μc	30	33.0	.50	3.3
$Pu^{239}O_2$ and dibenz[a,h]anthracene	.009 μc .05 mg	36	72.0	1.3	5.5

*Papillary cystadenoma
**Non-specific pneumonitis

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of injected material, 140 times more Ru^{106} than Pu^{239} was required to produce a 30 per cent tumor incidence. Although no data on the actual radiation dose to the lung are available,the apparent greater tumorigenicity of Pu^{239} as compared with Ru^{106} may be due to the higher RBE of alpha radiation (4).

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1086700

ABSORPTION OF STRONTIUM FROM FIELD PLOTS

J. F. Cline, A. A. Selders*, and F. P. Hungate

Barley grown in field plots for three successive years removes more Sr^{90} from the soil as the time between contamination and cropping increases. Cultivation and phosphate addition had little or no effect on Sr^{90} uptake in the third year.

A study was initiated in 1954 to study the uptake of Sr^{90} into plants grown under field conditions. Data for the first two years have been reported (1, 2). This year's experiment was designed primarily to evaluate the effect of time between contamination and cropping on the amount of Sr^{90} taken up by barley.

METHODS

The initial contamination, methods of planting, harvesting, and analytical procedures were described previously (1). As shown in Table 1, two of the plots which were contaminated with 1 mc of Sr^{90} in 1954 each received an additional 10 mc before planting time in 1956. Each of the eight plots was fertilized with 180 grams of NH_4NO_3, and one had an additional 50 grams of KH_2PO_4. All plots were surface irrigated with 24 milliacre inches of well water. Subirrigation was discontinued in 1956.

Movement of Sr^{90} through the soil was tested by cutting a vertical plane in a plot which had not been cultivated. Using care to avoid cross contamination, quarter-inch strips of soil were removed at specified depths down to nine inches below the surface.

RESULTS AND DISCUSSION

In 1955 it was reported that barley grown on plots contaminated in 1954 contained a relatively higher concentration of Sr^{90} than when grown

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on plots contaminated just prior to seeding in 1955. This was tested again this year by recontaminating an additional pair of plots so that plants could be grown in soil to which the major amount of Sr^{90} had been added in one of three successive years. As shown in Table 1, the results this year as consistent with those previously reported in that the per cent uptake by plants grown on soil contaminated in 1956 was less than that from soil contaminated in 1955, which in turn was less than that from soil contaminated in 1954. The data to date suggest that the uptake is doubled with each additional year of the Sr^{90} being in the soil. Obviously a maximum value must be reached but there is no sign of such a maximum as yet. Apparently, radio-strontium added to the soil did not become bound in insoluble and unavailable form with increasing time in the soil but instead it became more available to the barley. A similar increase in uptake with time was noted by Nishita, et al, for Cs^{137}, but they found a decreasing uptake of Sr^{90} in successive cropping of Ladino clover in flat culture (3).

Cultivation appeared to have much less effect on Sr^{90} uptake this year than last year. This does not seem to be due to movement of the active material in the soil since Table 2 shows that it is still largely in the surface layers of the uncultivated plots. Addition of phosphate caused no detectable difference in the uptake of Sr^{90}.

ACKNOWLEDGMENTS

We wish to acknowledge the technical assistance of C. H. Hemphill, M. G. Brown and W. W. Coleman.

TABLE 1

Summary of Results from Strontium Plots

Plot No.	Treatment*	Year and amount Sr^{90} applied		Veg. wt. in g	Seed wt. in g	Sr^{90} concentration $\mu c \times 10^{-3}/g$ Veg.	Seed	% Removed by plants
1	C	1 mc	1954	3930	1920	6.6	0.4	2.1
12	--	1 mc	1954	3965	1875	3.9	0.5	1.7
3	C	1 mc 9 mc	1954 1955	4855	1860	15	1.7	.8
5	C	1 mc 9 mc	1954 1955	4120	1865	18	1.5	.7
6	C, P	1 mc 9 mc	1954 1955	5045	1705	15	1.5	.8
8	--	1 mc 9 mc	1954 1955	3990	1955	21	2.1	.9
4	--	1 mc 10 mc	1954 1956	4550	1865	11	1.3	.2
7	C	1 mc 10 mc	1954 1956	3935	1815	12	1.4	.2

* C - Cultivated, P - Phosphate was applied.

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TABLE 2

Penetration of Sr^{90} into the Soil in Surface Contaminated Plot

Sample depth in inches*	$\mu c\ Sr^{90}/g$ soil
Surface	.21
1	.18
3	.012
6	0
9	0

* One-quarter-inch strips were used as samples at the prescribed depths.

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1086704

UPTAKE OF P^{32} BY PLANTS

A. A. Selders* and F. P. Hungate

Plants grown in soil or nutrient solution that contain P^{32} concentrate more P^{32} if modest amounts of carrier are added. Low pH also increases the uptake of P^{32} into plants. Concentration and pH affect the uptake of P^{32} and of fission products in the same general way.

The uptake into plants of the more important fission products has been evaluated by standardized procedures (1). Since all of these products are nonessential to plant nutrition it seemed desirable to compare the results with values obtained in a similar manner using an essential nutrient. Radio-phosphorus was selected for this study.

METHODS

Both nutrient solution techniques and soil culture by the Neubauer method were used. The methods for both have been described (2). In each experiment the concentration of P^{32} is constant for all parts of the experiment and the concentration ratios are derived from this value. Soil analyses for soluble phosphorus were done by the method of Piper (3) and for total phosphorus by the method of Consolazio, et al, (4).

RESULTS AND DISCUSSION

Figure 1 shows the effect of concentration of phosphate on the uptake of P^{32} in barley grown by the Neubauer technique. Addition of phosphate up to 250 μg of P/g of soil increased the absorption of P^{32}. Further additions depressed uptake and decreased yield. Chemical analysis of the native soil

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showed that it contained 30 μg of soluble P and 470 μg of total P/g of soil.

Figure 2 shows results obtained from nutrient culture studies in which the plants were initially grown on 0.001 μg P/ml of medium and then during the four days prior to harvesting they were exposed to the concentrations shown. The root values only partially indicate the effective concentration in the root environment (2) since comparative studies of uptake by living and dead roots show that living roots take up two times more P^{32} than do dead roots. Thus the root concentrations shown are probably twice as large as the adsorbed P^{32} would indicate.

When the roots of bean plants were killed or removed the uptake of P^{32} was slightly greater than in the intact plant. Table 1 shows the amounts of P^{32} taken into the roots, stems, and leaves of normal plants, of plants whose roots were killed in boiling water, and of plants whose roots were cut off. In one series an excess of carrier phosphate was also added. The cutting apparently damaged some of the conducting tissue since the uptake into cut plants was slightly less than into plants whose roots were intact but dead. There was no effect of the excess of phosphate on uptake of the radioactive phosphate.

The rate of uptake of P^{32} into plants was very similar to the uptake of Sr^{90}, with both showing concentration factors in excess of one. As with all the fission products studied (1) increased acidity causes increased absorption. Addition of carrier causes greater uptake of the radioactive isotope but a maximum effect was obtained with lower concentrations of phosphate than was required with most of the fission products. It seems clear that similar mechanisms are involved in the uptake of non-essential fission product ions and of the essential phosphate ion.

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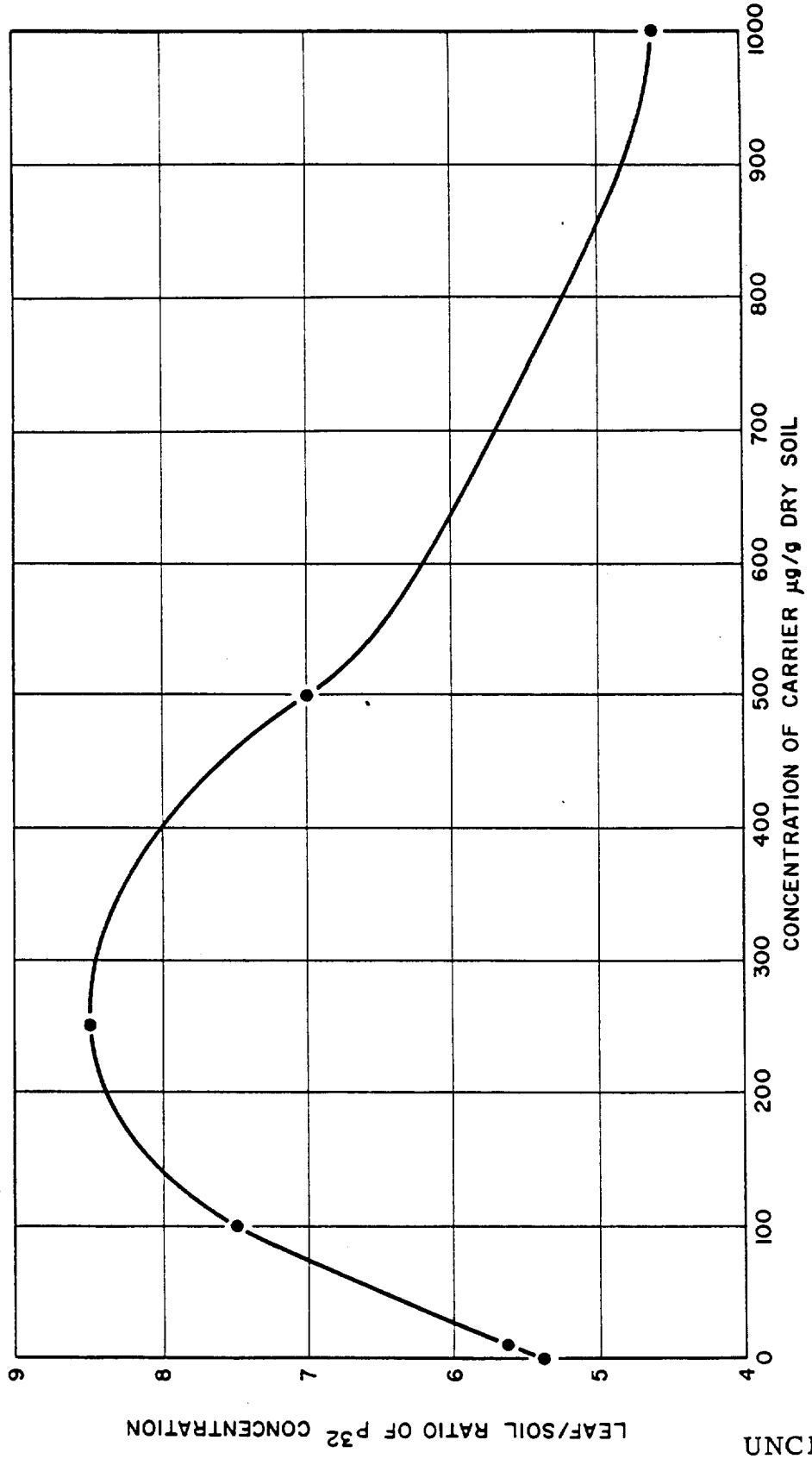

FIGURE 1

Effect of Carrier Concentration on P^{32} Uptake from Soil.

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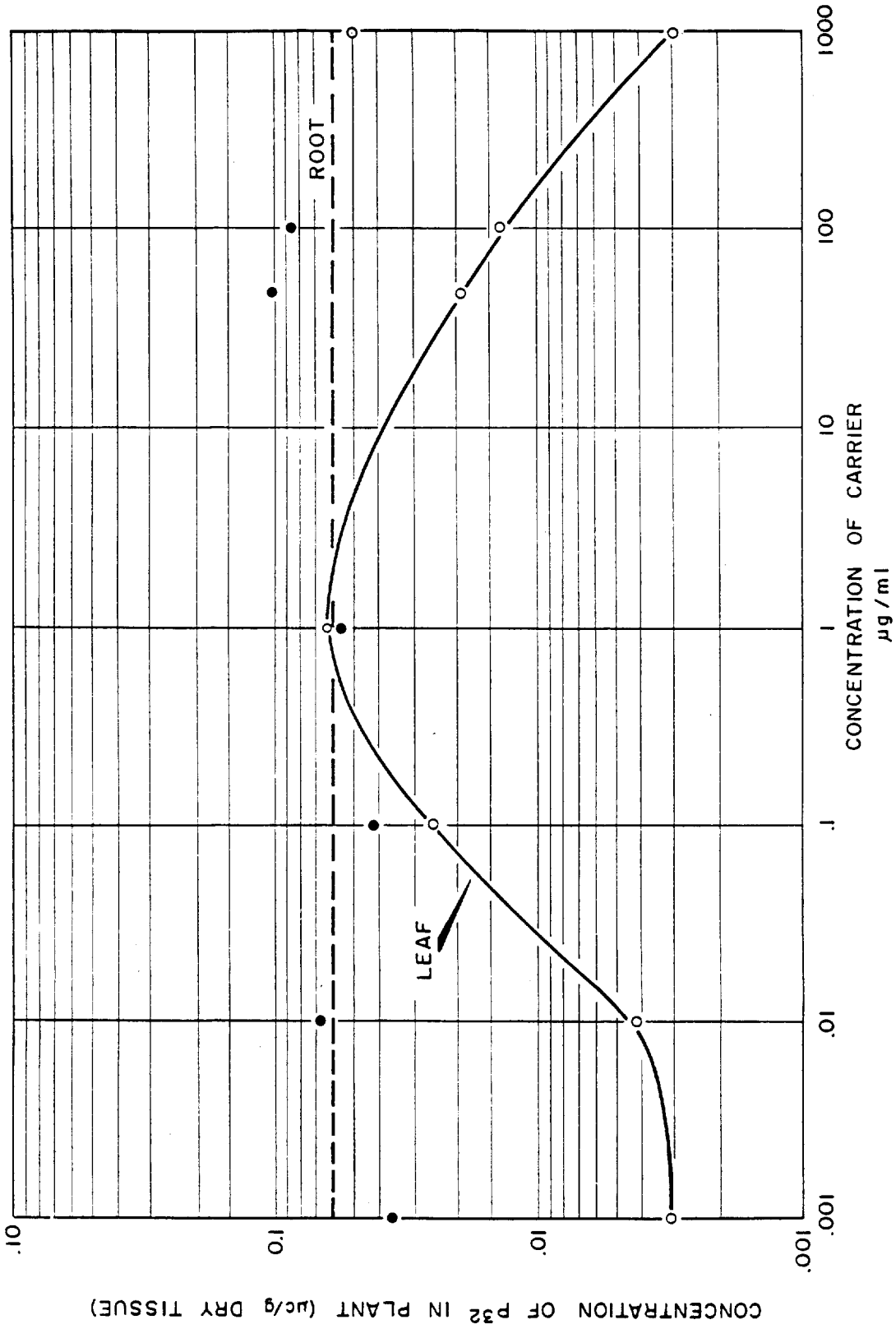

FIGURE 2
Effect of Carrier Concentration on P^{32} Uptake from Nutrient Solution.

TABLE 1

Uptake of P^{32} in Intact and "De-Rooted" Plants

Condition	P^{32} concentration, μc/g dry material		
	Leaf	Stem	Root
Control	0.6×10^{-2}	0.8×10^{-2}	3.3×10^{-2}
Hot H_2O	1.1×10^{-2}	1.5×10^{-2}	2.4×10^{-2}
Cut Off	0.7×10^{-2}	0.9×10^{-2}	1.6×10^{-2}
Hot H_2O + 100 μg P/g	1.2×10^{-2}	1.1×10^{-2}	2.3×10^{-2}

ACKNOWLEDGMENTS

We wish to acknowledge the technical assistance of C. H. Hemphill.

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1086709

BIOCHEMICAL MUTATIONS IN HAPLOID YEAST

F. P. Hungate and W. J. Bair

Over 200 biochemical mutants have been obtained by exposing haploid Saccharomyces cerevisiae to x radiation or culturing in medium containing S^{35}. The rates and types of mutants produced in several strains are compared.

Preliminary to a study of the genetic effects of internally deposited radioactive elements, several haploid strains of Saccharomyces cerevisiae were exposed to x radiation to establish techniques and to determine the kinds of mutations produced by ionizing radiation. One strain was also grown in medium containing S^{35} to compare the kinds of biochemical mutants produced with those produced following x radiation.

METHODS

For the x ray studies the yeasts were cultured at 30 C in complete medium containing Bacto-peptone and yeast extract. The cells were harvested by centrifugation and washed in distilled water. Distilled water suspensions in lusteroid tubes were exposed at room temperature to x rays from a General Electric Maxitron operating at 30 ma, 250 kvp, no added filtration, 20.5 cm TSD. The dose rate in air, unless otherwise noted, was approximately 10,000 r/minute. Following irradiation the suspensions were diluted and plated onto solid complete medium.

One strain, Sc-17-7-1, was also cultured in a chemically defined medium containing 100 μc S^{35}/ml. At the end of three days the suspended cells were added to tubes containing horse serum and lyophilized. After one to three months of storage at 5 C the tubes were opened and the yeast plated on complete agar.

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The replica plating technique of Lederberg (1) was used to make preliminary identification of heterotrophic mutants. Potential mutants from the replication test were isolated and tested for their specific requirements. Only those mutants are listed which in both tests grew on complete medium, but failed to grow on minimal medium.

RESULTS AND DISCUSSION

From the data in Tables 1 and 2 it is apparent that yeast can be utilized for studies of biochemical mutation. Most of the strains tested were about equally sensitive to the x rays, both in viability and frequency of mutation. There is an increased survival and reduced mutation rate in strain Sc-17-7-1. Whether this is related to the pantothenate requirement or is the result of other factors has not been ascertained since no crosses have been made to determine the genetic nature of this resistance.

It is interesting to note the effect of dose rate on viability and mutation frequency in cultures exposed to 302,000 r as shown in Table 1. Three hundred thousand roentgen given over a period of three hours had twice the killing effect and was ten times as mutagenic as the same dose given in half an hour. Since the cells were washed and irradiated while suspended in distilled water it is doubtful that there was any significant amount of cell division even in three hours. It is surprising that such differences in viability and in mutation might exist when the dose rate is relatively high in both parts of the experiment. No satisfactory explanation is apparent at the present.

The spectrum of mutant types obtained in these experiments is similar to that generally observed in studies with microorganisms. This is presumably due both to the method of testing and to biochemical similarities between organisms.

The frequencies of types of mutations obtained with S^{35} are slightly different from those obtained using x radiation. If this is an actual

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difference it may be due to different effects of x and weak beta radiation or it may result from the transmutation of metabolized S^{35}. This is presently under investigation.

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The technical assistance of Alys Harty and T. A. Kauth is gratefully acknowledged.

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TABLE 1

Relationship Between X Ray Dose and Mutation Rate

Yeast	x ray dose (r)	Per cent survival	No. of colonies tested	No. of mutants	Per cent mutation
Sc-10	Control	100.	241	0	---
	315,000	0.16	1425	55	3.8
Sc-13	Control	100	196	0	---
	315,000	0.13	865	33	3.9
Sc-10	Control	100	0	0	---
	283,200	1.2	88	3	3.3
Sc-10-12-4	Control	100	0	0	---
	105,000	1.7	114	0	
	210,000	0.64	600	6	1.
	420,000	0.1	125	7	5.6
	630,000	0.001	109	8	7.3
Sc-17	Control	100.	348	0	---
	142,800	0.3	1233	39	3.2
Sc-17-7-1	Control	100.	582	0	---
	80,000	19.	929	0	---
	160,000	14	1418	2	0.1
	320,000	5.6	1091	7	0.6
	640,000	0.1	391	19	4.8
	1,000,000	0.0005	53	0	---
Sc-17-7-1	Control	100.	738	0	---
	302,000*	6.0	1276	2	0.2
	302,000**	3.0	1222	21	1.7

Sc-10 obtained from H. Roman (ref. No. 275-IX)
Sc-13 obtained from H. Roman (ref. No. 1742A)
Sc-17 obtained from C. C. Lindegren (ref. No. 13894 - Pittman strain)
Sc-10-12-4 requires methionine, obtained by x-irradiation of Sc-10
Sc-17-7-1 requires pantothenate, obtained by x-irradiation of Sc-17

* 13,440 r/min.
** 2,124 r/min.

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TABLE 2

Numbers and Kinds of Heterotrophic Strains Produced

Requirement	Sc-10	Sc-13*	Sc-17	Sc-10-12-4**	Sc-17-7-1*** (x ray)	Sc-17-7-1*** (S^{35})
Inositol	6	2	16		--	--
PAB		4		1		
Thiamine	2	5		4	10	4
Biotin			1			1
Nicotinic Acid					1	
Choline						2
PAB or Biotin		1				
PAB or Thiamine		1				
Pantothenate			1		--	--
Arginine	3	6			1	8
Lysine	3	3			6	6
Methionine	6	7		--	2	4
Histidine	3	3		2	6	12
Tryptophane	6		2	1	3	4
Leucine	1					
Hypoxanthine						1
Cytosine	1					
Uracil and Cytosine		--	1		1	3
Uracil or Thymine or Cytosine		--				4
Adenine or Hypoxanthine (pink)	1	--			5	1
Adenine or Hypoxanthine (white)	3	--			3	6
Xanthine or Hypoxanthine					1	
Xanthine or Hypoxanthine					1	
Not determined	26	1	18	13	12	5

*Requires adenine and uracil
**Requires methionine
***Requires pantothenate (inositol was added to increase growth)

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1086715

THE EFFECT OF EDTA ON THE APPARENT RADIOSENSITIVITY OF YEAST

W. J. Bair and F. P. Hungate

Although Na EDTA decreased the uptake of plutonium, the inhibitory effect of plutonium on the growth of yeast was increased. Similar effects of EDTA with tritium show that the effect is not specific for plutonium but instead produces a synergistic effect with radiation delivered at low dose rates. The effect was not observed with x radiation delivered at high dose rates to non-growing cells.

The ability of calcium sodium ethylenediaminetetracetate (EDTA) to prevent the deposition and promote the excretion of administered plutonium has been observed in this laboratory and elsewhere. The present investigation was initiated to study the effects of EDTA on yeast cultured in the presence of plutonium.

METHODS

Growth studies were conducted using a diploid strain of *Saccharomyces cerevisiae* cultured at 30 C in an autoturbidimeter (1) which automatically recorded changes in the optical density of the suspension. Plutonium in 0.2 N nitric acid was added directly to sterile chemically defined medium. Sodium EDTA was used at a concentration of 3×10^{-4} M in all tests except those designed to evaluate effects from varying concentrations of this compound. All growth rate determinations were run in triplicate.

RESULTS AND DISCUSSIONS

The growth of yeast in the presence of plutonium and EDTA is shown in Figure 1. The increase in optical density of the cultures is

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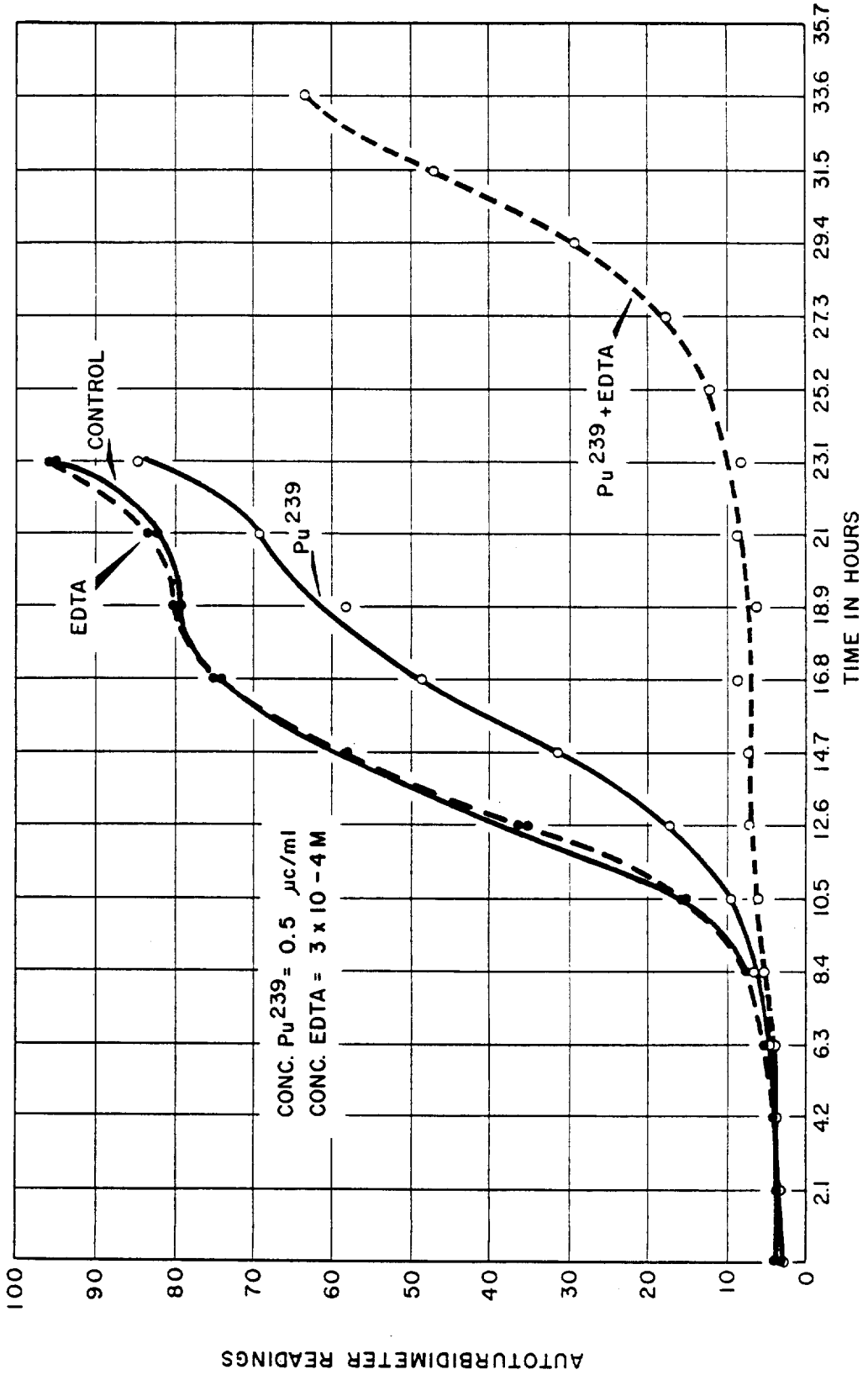

FIGURE 1

Growth of Yeast in Presence of Plutonium and EDTA.

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indicated by autoturbidimeter readings which are plotted against time. Growth in the control tubes was not altered by EDTA. Growth of yeast was delayed approximately three hours by 0.5 μc plutonium/ml. With EDTA also present a synergistic effect was observed and the inhibition of growth was much more pronounced.

Because other work in our laboratories had previously shown that EDTA was metabolized by yeast it appeared that this additional inhibition possible resulted from EDTA carrying chelated plutonium into the cells. This would cause a concentration of plutonium in the cells which consequently would receive a larger radiation dose than cells not concentrating the plutonium. That this supposition was not correct is shown in Table 1.

TABLE 1

Effect of EDTA on Uptake of Plutonium by Yeast

EDTA	Growing cells*		Non-growing cells**	
	Dry yeast mg/ml	Per cent Pu taken up	Dry yeast mg/ml	Per cent Pu taken up
None	3	70	1	58
3×10^{-4} M	2	1	1	41

*Growth medium contained 0.5 μc Pu^{239}/ml, pH 4.5.
**Two per cent glucose medium contained 0.1 μc Pu^{239}/ml, pH 3.

In a growing culture 3 mg yeast/ml removed 70 per cent of the plutonium from the medium. With EDTA present 2 mg yeast/ml took up only one per cent of the plutonium. This decreased uptake of plutonium in the presence of EDTA was also observed in non-growing suspensions of yeast. In two per cent glucose one mg yeast/ml removed 58 per cent of the plutonium. Only 41 per cent was taken up with EDTA present. From these data it is apparent that the effects from EDTA are not due to an increase in absorbed radiation dose in cells exposed to EDTA and plutonium.

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Rather, it appears that the effects from EDTA may be more extensive than indicated by the growth curves shown in Figure 1.

Since the EDTA appeared to augment the radiation effects from plutonium it was necessary to determine whether this was specific for plutonium or was a general synergistic effect with other than alpha radiation. For this investigation it was decided to use as a radiation source an isotope not likely to be chelated by EDTA or concentrated by the cell. Our earlier experience indicated that tritium would meet our requirements. Tritium oxide was added to the sterile medium and growth curves were determined as before.

Shown in Figure 2 are growth curves for yeast grown in the presence of tritium and EDTA. Again there was no evidence of an effect of EDTA on growth in the control tubes. Tritium, at 90 mc/ml of growth medium, produced a marked inhibition of growth. This was doubled by the synergistic effects of EDTA. Similar doubling of the inhibition was also observed at lower levels of tritium. The effect of EDTA thus appeared to be a general synergistic effect with radiation.

By employing higher concentrations of sodium EDTA it was possible to obtain a greater effect of EDTA on increasing the apparent radiosensitivity of yeast. However, higher concentrations of EDTA also inhibited growth in unirradiated control cultures. This is shown in Table 2.

The inhibition of growth is expressed as the additional time in hours required to attain a turbidity equivalent to an arbitrary turbidity reading of 50. No effect due to EDTA alone was observed until a concentration of 7×10^{-4} M was employed and growth was then delayed two hours. The inhibition due to 90 mc/ml tritium was four hours. The synergistic effect of EDTA and H^3 caused a delay of three hours when 3×10^{-4} M EDTA was present and five hours at the higher concentration of 7×10^{-4} M. Complete inhibition of growth occurred in 2×10^{-3} M EDTA.

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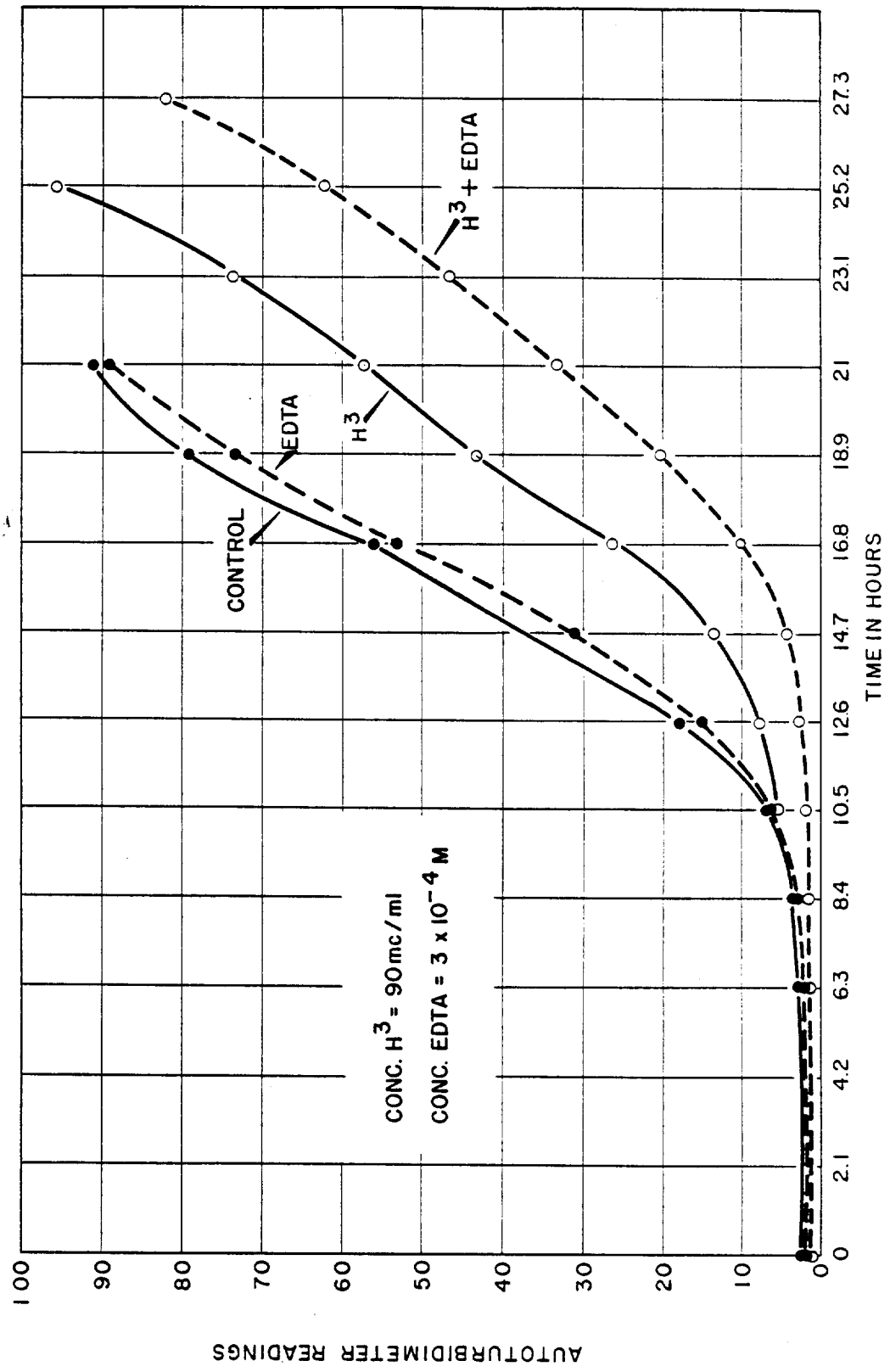

FIGURE 2

Growth of Yeast in Presence of Tritium and EDTA.

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TABLE 2

Effect of EDTA and Tritium on Growth of Yeast

EDTA	Hours delay* due to		
	EDTA	H^3**	Synergistic effect
0	0	4	0
3×10^{-4} M	0	4	3
7×10^{-4}	2	4	5
1×10^{-3}	30	very little growth	
2×10^{-3}	no growth		

*Hours delay in attaining a turbidity equivalent to a potentiometer reading of 50.

**90 mc H^3/ml.

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These results suggested that either radiation increased the sensitivity of yeast to EDTA or EDTA increased the sensitivity of yeast to radiation. If radiation increased the sensitivity of yeast to EDTA then exposure of yeast to x radiation with subsequent growth in EDTA should result in a decreased rate of growth. However, EDTA in the growth medium did not alter the inhibition of growth produced by a single exposure of the inoculum to 300,000 r. Also, incubation of yeast in EDTA for two hours prior to x radiation did not alter its ability to grow either in liquid culture or on complete agar. The latter is demonstrated in Table 3.

The survival of yeast following treatment was determined by its ability to form visible colonies on agar. Because it was impractical at the time to expose growing cultures to continuous x radiation the inoculum was exposed to rather large single doses of x radiation. Two different dose rates were employed to permit a closer correlation with the radioisotope experiments although the lowest x ray dose rate obtainable was still a hundred times greater than with tritium. Three per cent of the yeast survived when exposed to 300,000 r at 2,000 r/min and six per cent when exposed to 300,000 r at 13,000 r/min. No marked effect of EDTA on survival following x radiation was observed.

Our results are summarized in Table 4. Included also are calculated dose rates and published values for Linear Energy Transfer of the radiations used. It is apparent that the only marked differences between x ray experiments in which EDTA was ineffective in increasing the apparent radiosensitivity of yeast and the tritium and plutonium experiments in which EDTA was effective are dose rate and length of exposure. Also, in the x ray experiments non-growing cultures were irradiated.

There is insufficient data to suggest an explanation for the results. It is possible that the effects which were observed were due to the action of radiation on specific cellular processes rather than on reactive intermediates. The chromosomes may be the cellular processes involved.

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TABLE 3

Ineffectiveness of EDTA in
Altering Survival Following X irradiation

EDTA*	Dose Rate** (r/min)	Per Cent Surviving
None	Control	100.
	2,000	3.
	13,000	6.
3×10^{-4} M	Control	100.
	2,000	3.
	13,000	7.

*EDTA treatment - yeast suspension shaken for two hours prior to x irradiation.

**Total dose = 300,000 r, HVL = 0.25 mm Al, yeast suspended in H_2O at 25 C.

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TABLE 4

The Effect of EDTA on the Apparent Radiosensitivity of Yeast

Radiation	Dose rate per min.	Approximate LET values kev/μ	Inhibition of growth* due to	
			Radiation	Synergistic effect of EDTA**
X ray	10,000 r (for 10 min)	3	10	0
Beta (H^3)	18 rad	5	4	3
Beta (H^3)	9 rad	5	3	2
Alpha (Pu^{239})	0.09 rad	100	3	15

* Hours delay in attaining a turbidity equivalent to a potentiometer reading of 50.

** 3×10^{-4} M EDTA (No effect on growth in non-irradiated cultures.)

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The ability of EDTA to produce chromosome aberrations and to increase the rate at which aberrations are produced by radiation administered at low dose rates has been reported by others (2) as have also more generalized effects on ionic balance which in turn affect chromosome behavior (3).

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1086725

CHEMICAL TOXICITY OF PLUTONIUM IN YEAST

W. J. Bair and F. P. Hungate

Inhibition of glucose uptake and respiration of yeast by plutonium was observed. By the nature of the inhibition it is concluded that the inhibitory action was due to chemical toxicity.

Occasional suggestions that the toxicity of plutonium in animals is not entirely explained by radiation effects stimulated an interest in determining whether chemical toxicity may be involved. Rothstein observed inhibition of glucose uptake and respiration in yeast in the presence of 10^{-5} moles uranium per liter as uranyl nitrate (1). Because glucose uptake and respiration in yeast are known to be relatively insensitive to radiation (2) these appeared to be logical processes in which chemical toxicity of plutonium might be demonstrated.

METHODS

The yeast used was a diploid strain of Saccharomyces cerevisiae obtained from H. Roman, University of Washington. After culturing the cells overnight in a yeast extract and bacto-peptone medium they were washed, suspended in water, and starved by aeration for two hours prior to use in order to bring the yeast to a constant phase of endogenous metabolism (3).

Glucose uptake studies were conducted on yeast suspensions agitated in a water bath at 27 C. Aliquots were removed after two hours. The cells were separated from the medium by centrifugation and the supernatant analyzed for glucose and Pu^{239}. Respiration studies were by the direct method of Warburg (4).

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Plutonium (IV) in 0.23 N HNO_3 was used and added directly to a water suspension of cells followed in 10 minutes by the addition of substrates. The pH was adjusted by addition of HCl or NaOH. In respiration studies an equivalent amount of HNO_3 was added to the control flasks and the pH in both the control and plutonium containing suspensions was adjusted to approximately 5 with NaOH.

RESULTS AND DISCUSSION

Previous experiments had indicated a marked depression of yeast growth when the culture medium contained 3.2 μg Pu^{239}/ml and almost complete inhibition at 16 μg/ml. These data provided a basis for choosing plutonium levels in the present experiments. It was desirable to conduct the metabolic studies at a low pH in order to minimize the formation of colloidal aggregates.

The effect of plutonium on glucose uptake is shown in Table 1. At pH 2 to 3.5 an inhibition of 20 to 30 per cent was observed which appeared to be independent of plutonium concentration within the tenfold range of concentrations tested. No inhibition was observed when the yeast was buffered at pH 4.5. This may be due to the absence of ionic plutonium in solution (5). The reduced uptake of plutonium by yeast when buffered with triethylamine-succinate-tartrate is to be noted.

A similar inhibition of respiration by plutonium was observed. The data presented in Table 2 show an approximately 30 per cent depression in the rate of oxygen uptake.

These data are consistent with the premise that plutonium is chemically toxic to yeast because:

1. Glucose uptake was not found to be markedly altered by high doses of x radiation (6).
2. The maximum observed dose to the yeast in these experiments is less than that found to be necessary to depress respiration (7).

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3. Since the radiation dose was cumulative during the course of these experiments any inhibitory effects caused by radiation should have increased with time. Instead, the inhibition occurred immediately and no cumulative effects were observed.

The inhibition by plutonium appears to differ from that observed by Rothstein (1) for uranium. At comparable concentrations of 2 to 3×10^{-5} moles/liter, uranium showed 90 per cent inhibition while plutonium gave only 20 to 40 per cent inhibition. This difference is probably due to the difference in chemical properties of the two metals and the form of the ions used. Rothstein used the uranyl ion.

The lack of an effect of plutonium concentration on inhibition is not explainable at present. It suggests that the plutonium saturates the receptor sites in a limited area such as the cell surface but does not penetrate into the cell to produce internal poisoning.

ACKNOWLEDGEMENTS

The assistance of A. Harty and P. L. Hackett and J. M. Ryan of the Biological Analyses Operation is gratefully acknowledged.

1086728

TABLE 1

Effect of Plutonium on Glucose Uptake

Pu^{239} µg/ml	pH		Glucose Uptake		Pu^{239} uptake µg/mg yeast
	Initial	Final	mg/mg yeast	% Inhibition	
Control	3.5	3.5	1.00	-	-
3.0	3.5	3.2	.77	23	2.36
	3.5	3.5	.78	22	0.16
Control	2.2	<3	.95	-	-
2.5	2.2	<3	.70	26	-
Control*	6.0	4.2	1.93	-	-
2.59	2.0	2.5	1.27	34	2.23
0.21	3.	3.6	1.61	17	0.14
HNO_3 (0.23N)	2.	2.6	1.48	22	-
Control**	4.5	4.2	3.09	-	-
1.65	4.5	3.6	3.13	0	1.54
Control***	4.5	4.6	2.14	-	-
1.65	4.5	4.4	2.21	0	0.17

*pH not adjusted
**Buffered with M/15 KH_2PO_4
***Buffered with 0.02 M triethylamine-succinate-tartrate

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TABLE 2

Effect of Plutonium on Oxygen Uptake

Pu^{239} Concentration μg/ml	Time in hours	Q_{O_2}*		Per cent inhibition
		Control	Pu^{239}	
4.3**	1	37.9	25.8	31.9
	2	37.3	23.6	36.7
18.2***	1	39.6	26.2	33.8
	2	40.4	28.8	28.7
	3	41.7	32.9	21.2

Substrate was 1 per cent glucose.

Initial pH = 5 (no buffer).

*μl Oxygen/mg yeast dry weight/hour.
**0.6 mg yeast/ml.
***1.34 mg yeast/ml.

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1086731

ROLE OF FOOD HABITS IN WILDLIFE CONTAMINATION AT HANFORD

W. C. Hanson and R. L. Browning

Food items in stomachs of 522 wildfowl collected along the Columbia River and at waste ponds on the Hanford project were identified to correlate tissue concentrations of radioisotopes with wildfowl food habits.

Waterfowl which visit the Hanford reservation assimilate radioactive materials from food organisms which they obtain from the Columbia River or from waste ponds. As one of the measures taken to assure the contamination in game birds does not reach levels which would make them hazardous to off-project hunters, the important species are sampled at appropriate intervals as a part of the biological monitoring program (1). This sampling has shown that the concentration of radioactive materials is considerably different in the various species. Because such differences were presumed to result from different food habits, the stomach contents of the birds were examined to identify the plants and animals which act as vectors for radioactive materials.

METHODS

Stomach contents were placed in petri dishes, water was added to suspend the material, and food items were separated and counted under magnification. Plants were identified by comparing their seeds or other parts with reference specimens or texts (2, 3). Insects were identified from hard parts which resisted digestive processes. Abundance of any one food item was determined by complete counting of all individuals when few in number or by counting a representative fraction when many were present. The per cent of the total volume contributed by each item was visually estimated.

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RESULTS AND DISCUSSION

Relationship of Food Habits and P^{32} Concentrations in River Wildfowl

Relative importance of food items identified in stomachs of 421 birds collected along the Columbia River during 1956 are summarized in Table 1.

Relative P^{32} concentrations in tissues of eight major groups of birds utilizing the Columbia River during 1956 and their important dietary constituents are summarized in Figure 1. Because migratory habits influence their length of exposure to radiophosphorus-bearing biota of the Hanford project, the avian groups are listed in order of decreasing P^{32} concentration in three residential classifications: winter residents (January to March), summer residents (April to September), and fall migrants (October to December).

Birds which consumed significant amounts of insects consistently contained greater P^{32} concentrations than those whose diet was primarily fish. Swallows which fed upon adult insects exclusively contained lesser concentrations than shorebirds, whose diet included insect larvae. Birds which fed upon small fish generally contained greater P^{32} concentrations than those which preyed upon large fish. During the period June to August the food preference of mergansers shifted from large to small fish; the relative P^{32} concentration of this group of birds then exceeded that of gulls, which has shifted in feeding habit from large fish to adult insects and refuse. Evidence of such seasonal shifts in food preference among river wildfowl in general is inconclusive at the present time, although present data suggest such shifts may occur, especially during summer months. This further complicates the evaluation of the P^{32} concentrations of birds, which are subject to variation due to movement of the birds into and out of the sampling areas and by seasonal changes in the contamination level of the food organisms. Generally, the greatest concentrations of P^{32} occurred in those birds consuming food organisms with the highest level of

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TABLE 1

Major Food Items in Stomachs of Columbia River Wildfowl

Specimen type	Per cent of total volume of food items*								
	Algae	Immature insects	Adult insects	Small fish	Limpets and snails	Large fish	Crayfish	Vascular plants	Misc.
Shorebirds	-	15	55	9	20	-	-	-	-
Swallows	-	-	100	-	-	-	-	-	-
Diving ducks	3	57	5	1	17	-	1	8	8
River ducks	1	5	18	-	3	-	-	72	1
Grebes	5	9	15	55	2	-	6	-	8
Terns	-	1	29	70	-	-	-	-	-
Gulls	1	3	15	2	5	58	-	1	16
Mergansers	-	1	-	30	-	63	5	-	1

*Food items are arranged from left to right in decreasing order of P^{32} concentration. Because of different preferences by various birds insects are expressed as "adult" or "immature", and fish are arbitrarily divided into "large" (> 4" long) or "small" (< 4" long). Adults and immature stages of caddisflies (Trichoptera) were the insects consumed most often by birds other than swallows, in whose stomachs beetles (Coleoptera) and flies (Diptera) occurred in equal amounts.

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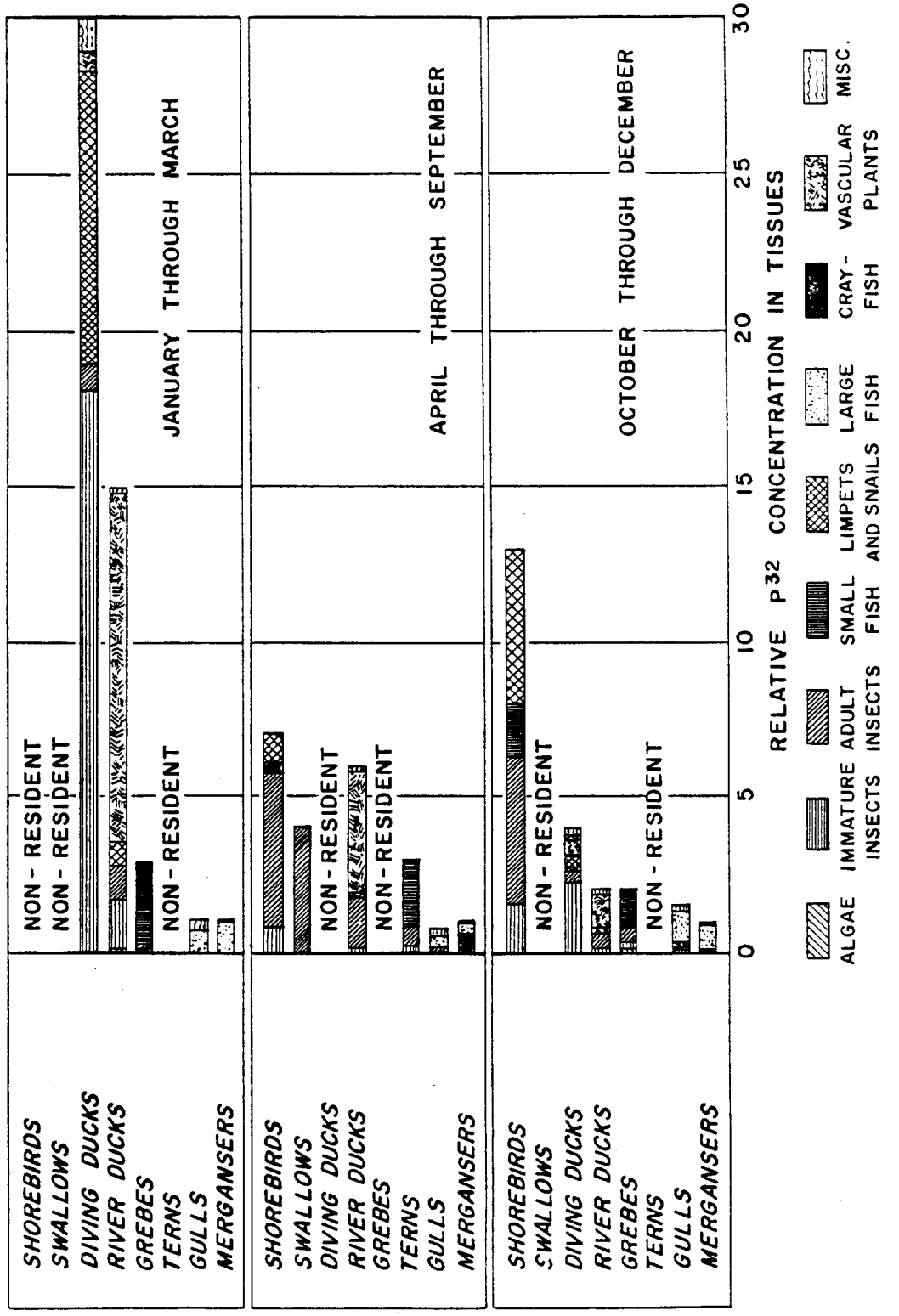

FIGURE 1
Relative P^{32} Concentration in Tissues

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contamination. Similar results were obtained by Davis, et al (4).

Relationship of Food Habits and Fission Product Concentration of Wildfowl at Waste Ponds

Algae, insects and vascular plants are available to wildfowl which frequent waste-water impoundments that contain traces of fission products. The seasonal importance of these items in the diet of coots, which are resident throughout the year, is illustrated in Figure 2. In general, usage reflects availability of the foods, especially seeds of higher plants. Diving ducks which consumed more insects than did coots or river ducks (Table 2), exhibited a greater uptake of such important radioisotopes as Sr^{90}, Cs^{137}, and Ru^{106}. During the final quarter of 1956, at which time the population of birds utilizing waste ponds became stabilized, tissue concentrations of fission products in diving ducks were six times those of coots and five times those of river ducks.

ACKNOWLEDGMENTS

The assistance of J. J. Davis and D. G. Watson of the Aquatic Biology Operation is gratefully acknowledged.

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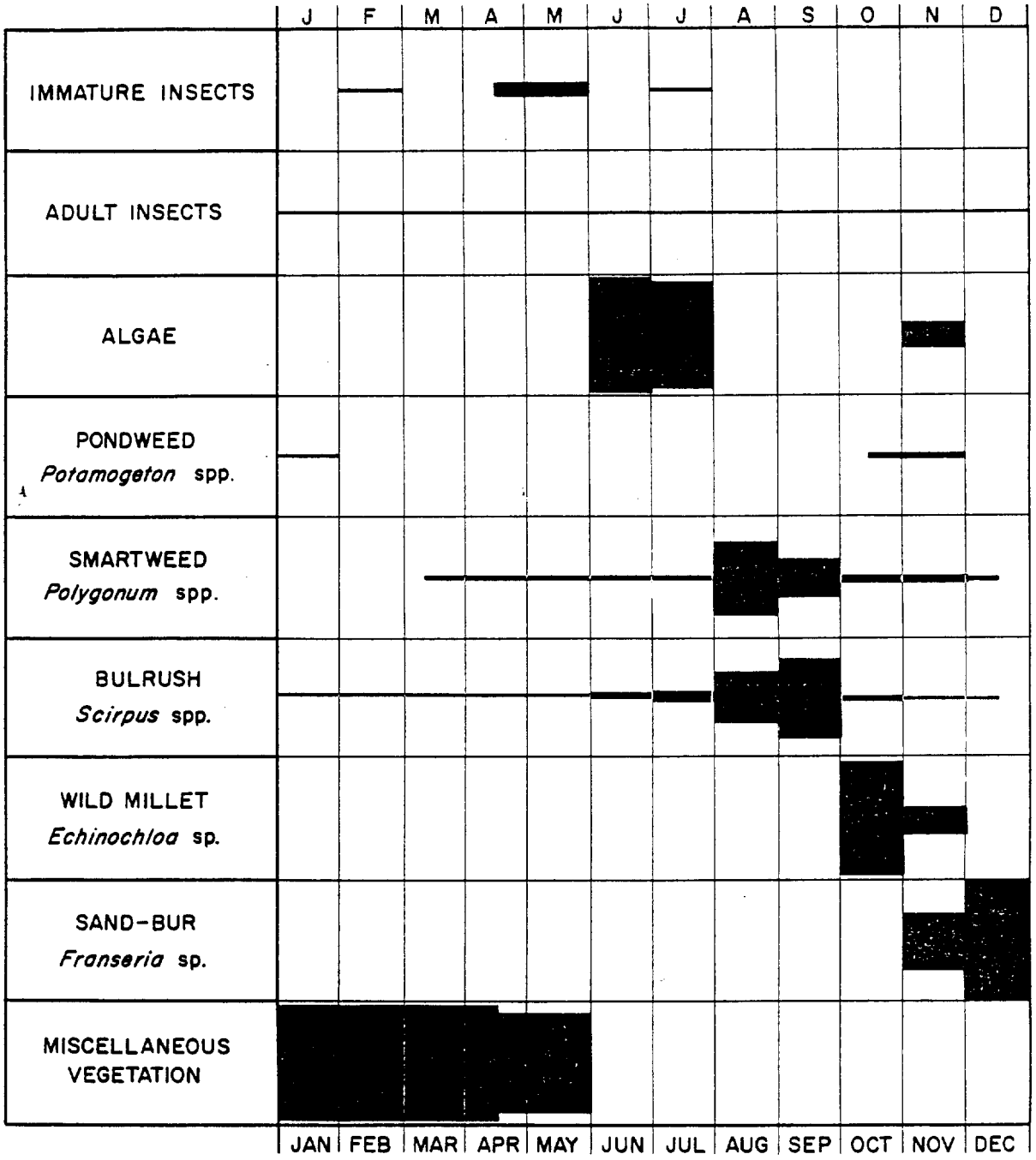

FIGURE 2

Seasonal Volumes of Food Items in Stomachs of Coots at Waste Ponds. Width of Bar in Proportion to Per Cent of Diet.

TABLE 2

Major Food Items in Stomachs of Wildfowl Frequenting Waste Ponds.

	Per cent of total volume of food items								
Specimen type	Immature insects	Adult insects	Algae	Pondweed	Smart-weed	Bulrush	Wild millet	Sand-bur	Misc. veg.
Diving ducks	12	31	-	10	7	11	1	-	31
River ducks	-	6	10	-	27	26	4	2	26
Coots	1	1	15	-	7	21	11	6	37

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1086739

ACCUMULATION OF RADIOISOTOPES IN RATS CHRONICALLY EXPOSED TO REACTOR EFFLUENT WATER

R. F. Palmer

A study is described which was designed to give a biological measure of the potential hazard of radioisotopes contained in reactor effluent water, under chronic exposure conditions. The P^{32} concentration in bone was higher than that of any other of the radioisotopes measured.

In an attempt to obtain a direct biological measure of the radioisotopes in Hanford reactor effluent water which are of greatest potential hazard under chronic exposure conditions, ten rats received concentrated reactor effluent water as their drinking water for as long as one year. Radiochemical analyses of the tissues from these rats were performed to determine which radioisotopes in the reactor effluent had been absorbed and deposited. As had been anticipated, the concentrations of P^{32} in both skeleton and soft tissue were higher than those of any of the other radioisotopes measured.

METHODS

Twenty adult female Sprague-Dawley rats weighing between 210 and 250 g were used in this study. For from six to twelve months, one-half of these animals received as their drinking water reactor effluent water concentrated an average of 33 times by volume. The other ten animals were provided drinking water whose composition approximated that of the concentrated reactor effluent except that it contained no radioisotopes. Concentration of the reactor effluent water was effected in acid solution to prevent excessive loss of radioisotopes to the walls of the concentrator. The final solution for animal feeding was adjusted to pH 1.5 which was acceptable to the animals and still retained the solids in solution.

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The animals were killed by decapitation under ether anesthesia, skinned, and the G. I. tracts and feet removed. After autoclaving, each animal was divided into two samples: skeleton and soft tissue. This latter included muscle, heart, lungs, liver, kidneys, and spleen. Each sample was digested in nitric acid until all organic matter was destroyed. Aliquots were assayed for radioisotopes of phosphorous, strontium, barium, cesium, and mixed rare earths by standard radiochemical techniques. Low-background proportional counters, (background of 0.67 to 1.86 c/m) were used to measure the low concentrations of beta-particle emitters in the various samples. Decay curves are being obtained to confirm the identity of the separated radioisotopes.

RESULTS AND DISCUSSION

Based on results not corrected for possible contaminants that may be revealed when decay studies are completed, the average radioisotope concentrations in the experimental animals at the time of sacrifice are given in Table 1.

TABLE 1

Radioisotope	Concentration at Sacrifice ($\mu c/g \times 10^5$)	
	Bone	Soft Tissue
P^{32}	8.9	1.6
Rare earths	1.4	<0.1
Ba^{140}-La^{140}	0.5	<0.1
Cs^{137}	0.4	0.4
Sr^{89}, Sr^{90}-Y^{90}	0.3	<0.1

These concentrations are all only small fractions of the maximum permissible body burdens recommended by the I.C.R.P. (1)

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As decay studies are still in progress on most of the samples, it is not possible to report final results at this time. It is evident from the results in hand that the isotopes measured did not deposit in bone to the extent one would predict on the basis of their concentrations in the drinking water and the presently accepted values for absorption and distribution coefficients.

Supplemental studies, in which rats were fed P^{32} added to reactor effluent water before and after its concentration, have shown that the absorption of P^{32} is not appreciably affected by the presence of the concentrated reactor effluent or by the process of concentration. Preliminary experiments comparing P^{32} absorption and deposition in bone of year-old rats and in younger animals were also conducted. The results suggest that the lower than predicted build-up of the various isotopes measured in the animals maintained for one year on concentrated reactor effluent may have been due to the advanced age of the animals by the end of the experiment. More extensive experiments to resolve this question are planned.

Analyses of bone and soft tissue from the control animals used in this study showed no radioactivity that could not be attributed to the natural K^{40} content of the animals.

ACKNOWLEDGMENTS

The able technical assistance of J. T. Homer is gratefully acknowledged, and sincere thanks are extended to J. M. Nielsen and W. B. Silker of the Analytical Chemistry Operation for making available the low-level beta counting facility.

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1086742

CHRONIC EFFECTS OF REACTOR EFFLUENT WATER ON CEREAL PLANTS

J. F. Cline, A. A. Selders*, F. P. Hungate

No effects have been observed on the yields of seed vegetation, seed germination, total seed nitrogen, or mutation rates in barley irrigated with reactor effluent water for five years. The increase in content of radioactive material in the plants was barely perceptible. Concentrating the constituents of reactor effluent in the soil twenty-five times produced no visible effects on barley grown in the soil.

This report summarizes the fourth and fifth years' study of the chronic effects of reactor effluent water on the barley plant. Data for the first three years were previously reported (1, 2, 3). Seed obtained from the previous barley crops were planted each year.

METHODS AND RESULTS

Methods of watering, harvesting, and analytical procedures were generally the same as those previously reported (1).

No chronic effects were observed on the yields of seed or vegatation, seed germination, total seed nitrogen, or mutation rate, in the barley irragated with reactor effluent water for five years. Less than twice as much radioactive material was present in vegetation grown on plots irrigated solely with effluent water as compared with barley from plots irrigated with well water.

A reduced yield of seed and vegetation in 1955 was uniform in all plots and reflected climatic conditions of the area.

Constituents of reactor effluent water were concentrated in the soil twenty-five times by successively adding and then evaporating off 25 ml of

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effluent per gram of soil in an oven at 70 C. No visible symptoms of toxicity or effects on vegetation yield were observed in barley grown in this soil by the Neubauer technique.

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1086744

FURTHER STUDIES ON THE EFFECT OF SODIUM DICHROMATE ON JUVENILE CHINOOK SALMON

P. A. Olson and R. F. Foster

Chinook salmon eggs were not affected by concentrations of sodium dichromate which are lethal to the young fish after they have hatched from the eggs. When young chinook salmon were exposed to toxic quantities of dichromate, the effect was somewhat more severe when addition of the chemical was continuous than when an equivalent amount was added intermittently. Young salmon held in water at 41 F showed less tolerance for dichromate than salmon held in the more optimum temperature of 50 F.

Strong concentrations of effluent from the Hanford type reactors are toxic to sensitive fish. This toxicity has been attributed to the presence of chromium (1). Previous studies (2) showed that chronic exposure of eggs and fry of young salmon to about 0.1 ppm Cr(VI) resulted in high mortality, but it was not known whether: the eggs were as sensitive to the chromium as were the young fish (no significant mortality occurred until after the eggs hatched); intermittent exposure to high concentrations would affect the young fish as greatly as constant exposure to equivalent amounts at lower concentrations; within the tolerable range, higher temperatures aggravated the toxic effect. This paper describes tests which were run to provide answers to these questions.

MATERIALS AND METHODS

Aqueous solutions of commercial grade sodium dichromate were added to troughs supplied with water from the Columbia River and stocked with chinook salmon eggs or young. The chromium concentrations in each of the experimental conditions and in the controls were determined from water samples collected each week (3). The means of the analytical results were used as the best estimates of exposure conditions.

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The pH varied from 7.1 to 8.1. Dissolved oxygen was at or near the saturation level. Hardness was about 70 ppm. Temperature (except where controlled) changed with the normal seasonal variations of the Columbia River.

Mortality and rate of growth were used as criteria of damage. Samples of fish from each trough were weighed at intervals of approximately two weeks and the lengths of at least 100 salmon from each lot were measured at the end of the tests.

TOLERANCE OF EGGS TO SODIUM DICHROMATE

In a previous test (2), salmon eggs and subsequently the young fish were exposed to 0.18 ppm Cr(VI) for seven months. Although over 90 per cent of the young fish died, mortality during the egg stage was not significantly above normal. To determine whether the eggs actually had a high tolerance for the chromium or whether there was a latent effect, newly spawned chinook salmon eggs were exposed to sodium dichromate until hatching. Approximately 2300 eggs were randomly distributed between two troughs. One trough received Cr(VI) and the other served as a control. When the eggs began to hatch, 42 days after the start of the test, the dichromate addition was stopped. Observations on the two troughs of salmon were continued for an additional 16 weeks until the fish were ready to migrate to the ocean. During the test, temperatures ranged from a maximum of 59 F to a minimum of 35 F.

Table 1 shows the chromium concentrations in each of the experimental conditions.

TABLE 1

Chromium Concentrations During Egg Stage

ppm Cr(VI) added	Mean of weekly analyses ppm Cr(VI) $\pm \sigma$	Equivalent as ppm $Na_2Cr_2O_7 \cdot 2H_2O$
0	0.011 ± 0.002	0.03
0.17	0.174 ± 0.015	0.50

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Mortalities and growth experienced during the experiment are shown in Figure 1. There was no increase in mortality resulting from the exposure of the eggs to the 0.17 ppm. The number of deaths recorded for the exposed lot was actually less than for the control, but such a difference can be attributed to biological variability. At the end of the test the size of the fish in the two lots was virtually identical.

The results of this test show that salmon eggs can tolerate levels of dichromate that are fatal in later stages of development.

EFFECTS OF INTERMITTENT VERSUS CHRONIC EXPOSURES

To determine the relative effect of intermittent exposure to Cr(VI), about 2600 newly-spawned eggs were placed in each of three troughs under conditions shown in Table 2. The experimental design was such that each lot received the same total amount of Cr(VI) over a two weeks' period. The experiment was continued for seven months until the young fish, which hatched from the eggs, were of sufficient size and age for migration to the ocean.

TABLE 2

Chromium Concentrations During Test

Scheduled addition of Cr(VI) ppm	Mean of weekly analyses ppm Cr(VI) ± σ	Equivalent as ppm $Na_2Cr_2O_7 \cdot 2H_2O$
0.07 (continuous)	0.081 ± 0.030	0.23
0.14 (alternate weeks)	0.150 ± 0.039	0.43
0.49 (One 24-hour period per wk.)	0.505 ± 0.101	1.45

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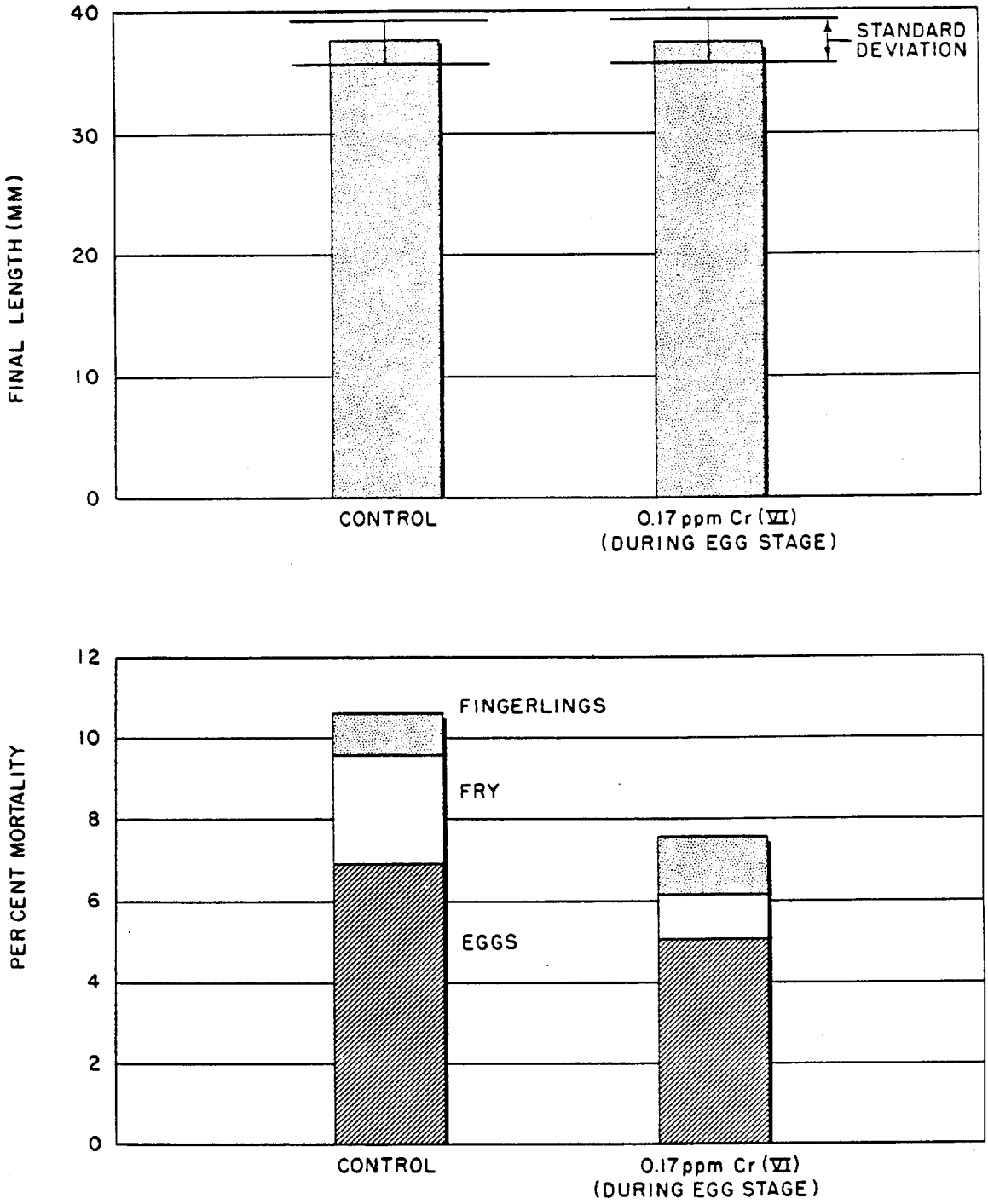

FIGURE 1

Effect of Exposure of Salmon Eggs to Cr(VI) on Mortality and Growth of the Fingerling.

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Mortalities experienced during the progress of the experiment are shown in Figure 2. No significant difference in mortality (5 per cent level of probability) occurred during the egg stage. After hatching, approximately six weeks passed before the death rate began to increase. At the end of the fry stage poorest survival was in the lot which received 0.15 ppm (alternate weeks) and best was in the lot which received 0.50 ppm (one 24-hour period per week). At the conclusion of the test, significantly fewer fish survived in the 0.08 ppm (continuous), and best survival was in 0.50 ppm (one 24-hour period per week). The flattening of the mortality curves during the last weeks of the exposure probably indicates survival of the more resistant individuals and also an increased resistance at this age.

Figure 3 shows the growth rates of the fish. At the end of the experiment the smallest fish were in the chronically exposed lot and the largest were in the lot exposed for 24 hours each week. The results are thus in agreement with the mortality data.

These results indicate (for this experimental design) that chronic release of dichromate is slightly more toxic to aquatic life than if the same quantity were released intermittently.

EFFECT OF TEMPERATURE ON TOXICITY

To determine whether the toxicity of dichromate varies with temperature within the tolerable range, fingerling-size (0.5 gram) chinook salmon were exposed to 0.09 ppm Cr(VI) at temperatures of 41 F (5 C) or 50 F (10 C) for a period of 74 days. The experimental design is shown in Table 3. A total of 800 fish was used in the study. The concentration of dichromate used was known to be toxic if administered for several weeks (2). The experiment was stopped at the end of May when the capacity of the water-chilling units could no longer maintain the 41 F temperature.

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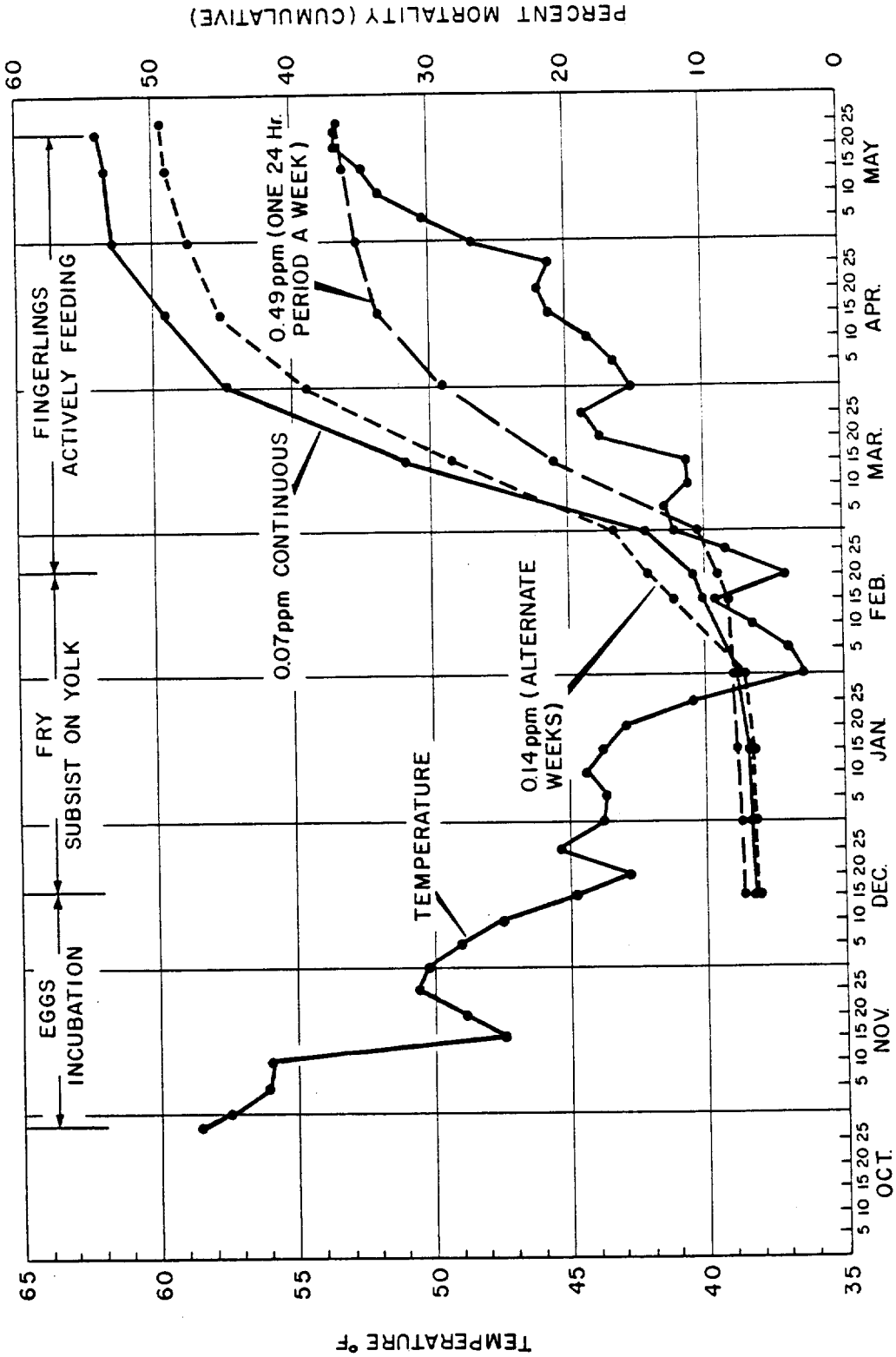

FIGURE 2

Effect of Intermittent Exposure to Cr(VI) on Mortality of Salmon.

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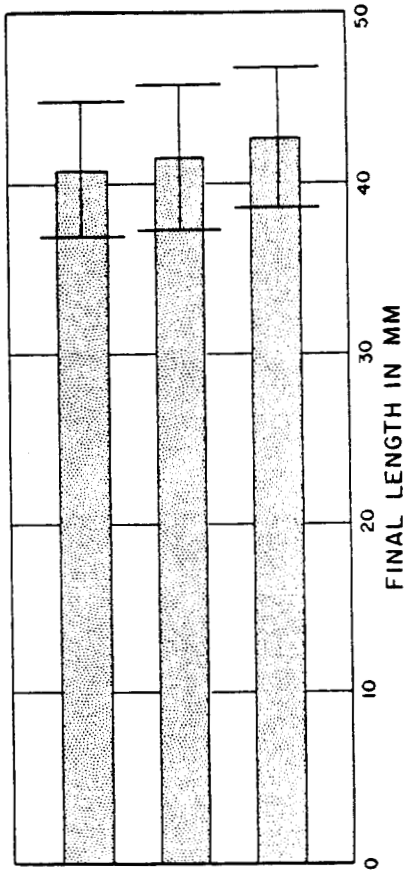

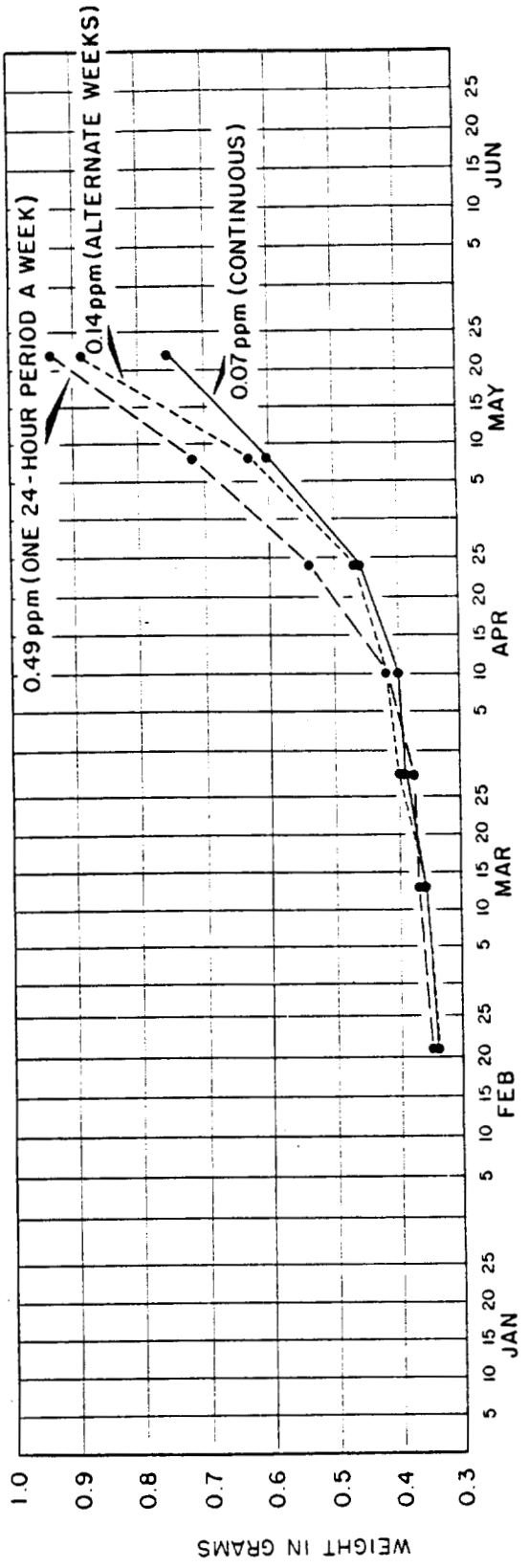

FIGURE 3

Effect of Intermittent Exposure to Cr(VI) on Growth of Salmon

TABLE 3

Chromium Concentrations During Test

Temperature	Mean of weekly analyses ppm Cr(VI) $\pm \sigma$	Equivalent as ppm $Na_2Cr_2O_7 \cdot 2H_2O$
41 F	0.091 ± .018	0.26
41 F	0.004 ± .003	0.012
50 F	0.095 ± .023	0.27
50 F	0.004 ± .003	0.012

Although the temperatures selected were thought to be within the range which can be tolerated by chinock salmon of this age (4), there was no question but that 50 F was more favorable than 41 F. The fish held at 50 F appeared to be in better condition and were more active. Fewer fish died at 50 F, but the difference was not statistically significant. Figure 4 shows the mortalities which occurred in each of the lots. Only among fish exposed to 0.09 ppm Cr(VI) at 41 F was the mortality significantly above normal.

Figure 5 shows the growth rate of the fish in each lot. Comparison of the rates of growth between the two temperature conditions has little meaning in this test since small differences in temperature affect metabolic rates and thus the rates of growth. The presence of 0.09 ppm Cr(VI) did, however, markedly retard growth at each temperature level.

These results suggest that dichromate has a greater toxic effect at 41 F than at 50 F. No simple correlation between temperature and toxicity is indicated, however. It seems probable that fish which live in the most optimum temperature condition are best able to tolerate the toxicity of dichromate.

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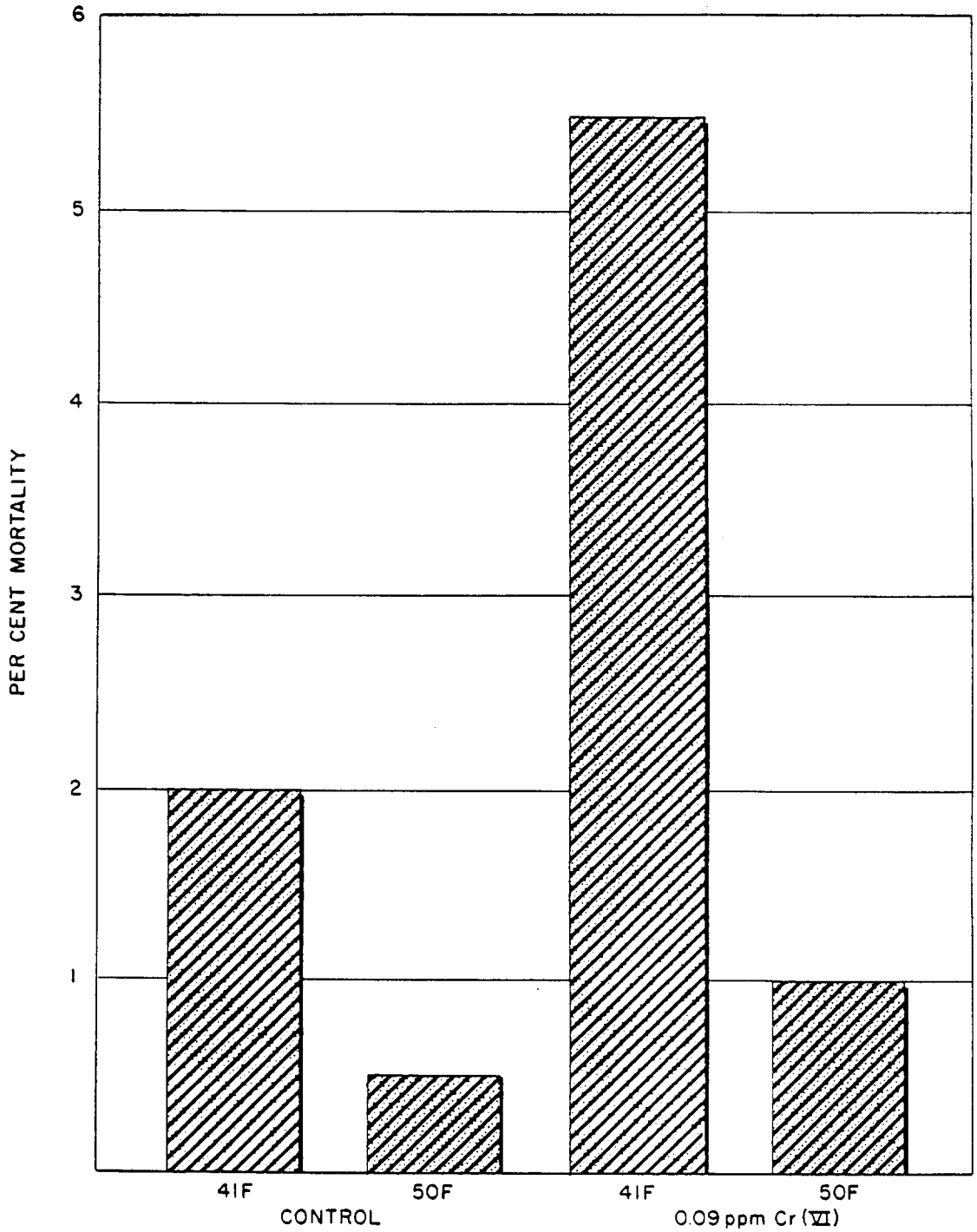

FIGURE 4

Effect of Cr(VI) and Temperature on Mortality of Chinook Salmon Fingerlings.

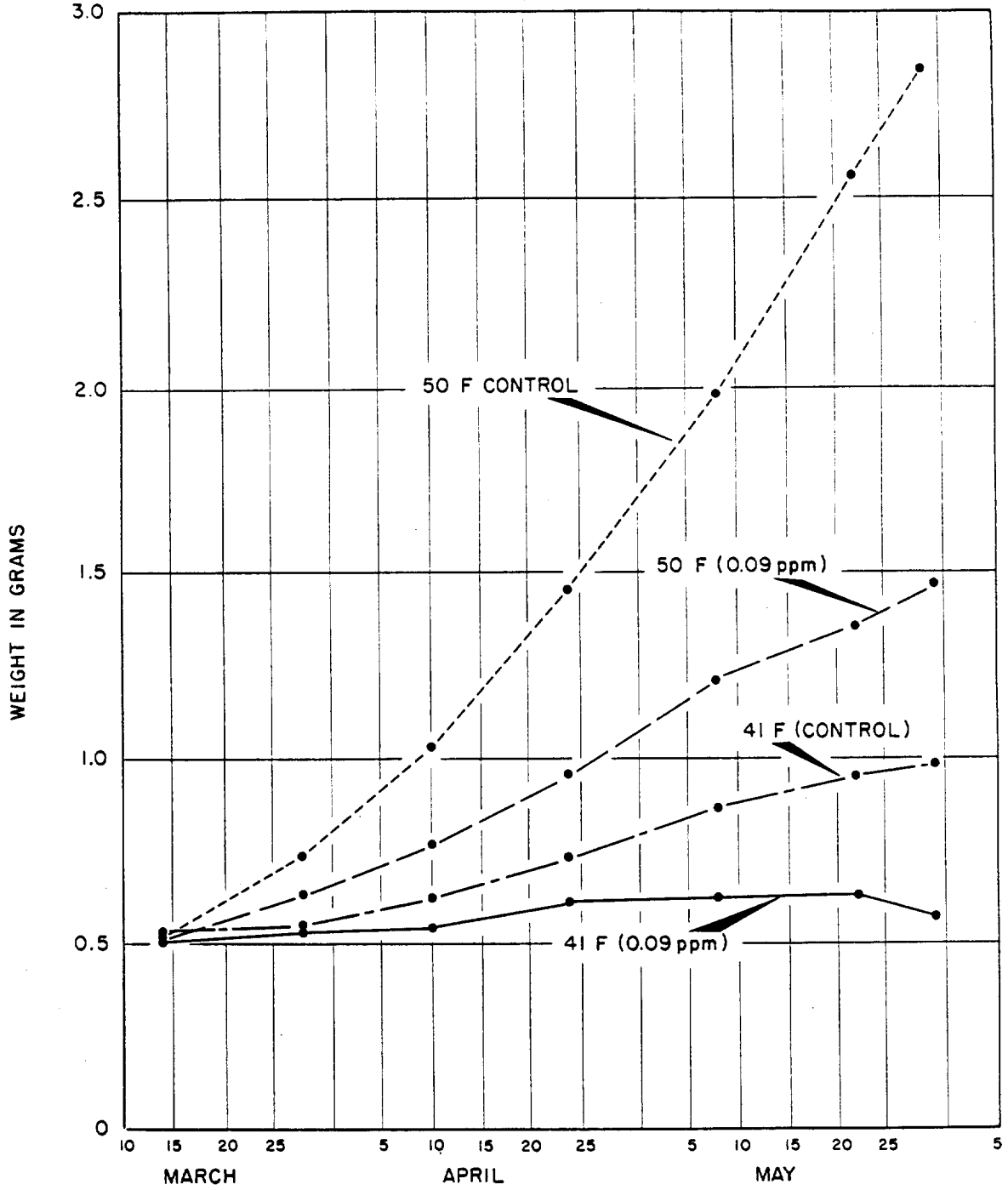

FIGURE 5

Effect of Cr(VI) and Temperature on Growth of Chinook Salmon Fingerlings

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ACKNOWLEDGMENTS

We are grateful to the Washington State Department of Fisheries for providing the eggs used in these tests and to Dr. L. R. Donaldson of the University of Washington for assistance in procuring them. We also wish to express our thanks to A. C. Schroeder and the Biological Attendants who cared for the fish and operated the equipment and to Patricia Hackett and personnel of the Biological Analyses Operation for the chemical analyses.

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1086755

CHINOOK SALMON SPAWNING IN THE VICINITY OF HANFORD - 1956

D. G. Watson

The tenth in a series of annual surveys of the extent of chinook salmon spawning in the Columbia River near Hanford was made during the fall of 1956. Methods and results of years prior to 1956 have been presented in previous annual reports (1, 2).

A comparison of the nest counts in local spawning areas with the fall runs of chinook salmon ascending Bonneville Dam is given in Table 1. In 1956 the numbers of fish at both locations were below the average for the past ten years. In 1955 and 1956 the decrease in local spawning was greater than the decrease in the fall run passing Bonneville Dam. Because of the multitude of factors which affect the survival of salmon between the time the eggs are laid and their return as adults to the spawning grounds, no single factor can be assigned as the cause of the decrease.

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TABLE 1

Year	No. of Surveys	Nests per Major Spawning Areas						Total No. of Nests	Total No. of Fall Chinook Passing Bonneville Dam Aug. 16-Dec. 31	Nest Counts Near Hanford Per Cent of 10 Year Ave.	Fall Run Ascending Bonneville Dam Per Cent of 10 Year Ave.	Nest Counts near Hanford Per Cent of Brood Year *	Fall Run Ascending Bonneville Dam Per Cent of Brood Year *
		Miles from Mouth of Columbia River					Other						
		354 (Near Ringold)	365	369 (White Bluffs)	371 to 372	393 (below) Priest Rapids)							
1947	2	0	15	25	10	75	155	280	296,935	94	167		
1948	1	100	300	38	219	25	53	735	305,623	248	172		
1949	4	45	50	6	195	1	33	330	169,388	111	95		
1950	2	19	35	36	144	44	14	292	242,913	98	136		
1951	4	5	10	45	151	95	18	324	131,739	109	74	116	44
1952	4	73	101	40	221	78	32	545	214,288	184	120	74	70
1953	4	0	5	16	38	83	7	149	97,335	50	55	45	58
1954	3	4	5	8	127	6	7	157	100,499	53	56	54	41
1955	3	0	12	0	47	4	1	64	95,157	22	53	20	72
1956	3	0	3	7	59	17	6	92	125,983	31	71	17	59
Average	3	25	54	22	121	43	33	297	177,986				

* Assumes a 4 Year Life Cycle

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EFFECT OF MASSIVE DOSES OF P^{32} ON TROUT

D. G. Watson

The effects of feeding P^{32} to yearling trout at rates of 600, 3,000, and 3,800 microcuries each week for 12 weeks are described. All fish fed the two higher levels died of radiation damage within seven weeks, after receiving approximately 12 millicuries of P^{32}. Severe breakdown of the vascular system was evident at death. About 55 per cent of the ingested P^{32} was retained by the trout.

This paper describes a preliminary study on the effects of P^{32} on trout. The purpose of the study was to determine: the amounts of P^{32} necessary to produce death, the concentration of P^{32} in the tissues and organs at time of death, the gross damage produced, and the fraction of the ingested dose which was taken up.

METHODS

Four groups of yearling rainbow trout with an initial mean weight of 200 grams were used in this experiment. There were 12 fish in Group I, the controls, and 19 fish each in Groups II, III, and IV, the experimental lots. Groups II, III, and IV were fed P^{32} at an average rate of 600, 3,000, and 3,800 microcuries, respectively, each week for 12 weeks or until they refused food. The controls received phosphorus at the same rate as the group receiving 3,800 microcuries per week, but in the stable form. In order to administer the P^{32} without handling the fish, radiophosphorus was first injected into freshly killed fingerling trout which were then fed immediately to the experimental animals. The trout were fed P^{32} twice weekly and were given supplemental feedings of beef liver.

One fish from each of the irradiated groups was killed on the first, second, fourth, sixth, and ninth weeks of the experiment and the concentration of P^{32} in its tissues measured. In addition, one fish from each

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group was killed on the second and fourth weeks and the total amount of P^{32} in the body measured.

RESULTS AND DISCUSSION

All groups fed P^{32} exhibited changes in behavior and appearance attributable to radiation damage. They became very nervous four days after the initial feeding, but their behavior returned to normal within a week. A loss of scales roughly proportional to the amount of P^{32} received was also noted.

The two groups fed at the high levels showed almost identical symptoms, which included reduced appetite, listlessness, refusal to eat, darkening of the skin, gulping of air, and loss of equilibrium. Animals in the low-level group suffered some loss of appetite at 8 weeks but were nearly back to normal at 10 weeks.

Damage to visceral organs was evident in fish killed three days after the first feeding of P^{32}. All groups showed a marked increase in the size of the gall bladder that was approximately proportional to the amount of P^{32} ingested. This condition was also apparent at 10 days. At 20 days necrosis of the stomach lining, inflammation of the gut, and complete collapse of the gall bladder were typical of the two high level groups. Hemorrhage in the liver was noted at 24 days in these groups.

All fish fed P^{32} at the rate of 3,000 and 3,800 microcuries per week died within 68 days and 81 days respectively. Amounts of P^{32} ingested and time lapse until death are given in Table 1. The radiation syndrome at death was much the same for both these groups and included necrosis and hemorrhage of the gut, collapse of the gall bladder, and an extensive breakdown of the vascular system as exemplified by severe hemorrhage in the liver, spinal canal, gonads, and musculature (particularly that closely associated with the skeleton and fins).

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TABLE 1

Time of Death and Amount of Isotope Ingested by Rainbow Trout Fed Lethal Amounts of P^{32}

Group	mc P^{32}/kg/wk	Time of Death				Mc P^{32} ingested			
		Days after start of experiment		Days after last feeding of P^{32}		Per fish		Per Kg of fish	
		Mean	Range	Mean	Range	Mean	Standard deviation	Mean	Standard deviation
III	15	42	36-49	14	5-18	11.7	± 3	59	± 13
IV	24	33	28-36	13	10-17	12.3	±2	78	± 20

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There were no mortalities in fish fed 600 microcuries of P^{32} per week although gross damage was evident. This included enlargement of the gall bladder, hemorrhage in the liver and musculature and a thickening of the gut wall that produced, in some instances, a partial constriction near the junction of the mid and hind gut.

The histopathology of the tissues has not yet been studied.

Before radiation damage became severe an average of 54 per cent (range 43 to 70 per cent) of the administered P^{32} was retained by the trout. This is in reasonable agreement with data reported by Hayes and Jodrey (1) which indicated a retention of 66 per cent in trout after a single injection of P^{32} into the coelomic cavity. Whittaker (2) found that young salmon retained approximately 70 per cent of the P^{32} consumed, when fed Daphnia that contained radiophosphorus.

The concentration of P^{32} in various tissues at the time of death is presented in Figure 1. Wide ranges in uptake may be attributed to differences in the utilization of phosphorus between individual fish and to differences in amounts of P^{32} ingested before death occurred.

The radiation dose to bone, the tissue of maximum concentration of P^{32}, was about 60,000 rad and 74,000 rad respectively for Groups III and IV. Fish in Group II received on the order of 53,000 rad to the bone during a 16-week period. Kidney, the principal blood-forming organ in fish, was shown by Bonham et al, (3), to be severely damaged in chinook salmon by single high doses (2,500 r to 5,000 r) of x rays. The kidneys of trout in Group III and IV received doses of approximately 12,000 to 13,000 rad respectively. These values were calculated on the basis of the concentration of P^{32} in the kidney tissue. The additional dose received because of its close proximity to the bone was not included. The visceral organs in general received doses on the order of one-tenth that of bone. The gut, however, received considerably more than this amount due to the method of isotope administration.

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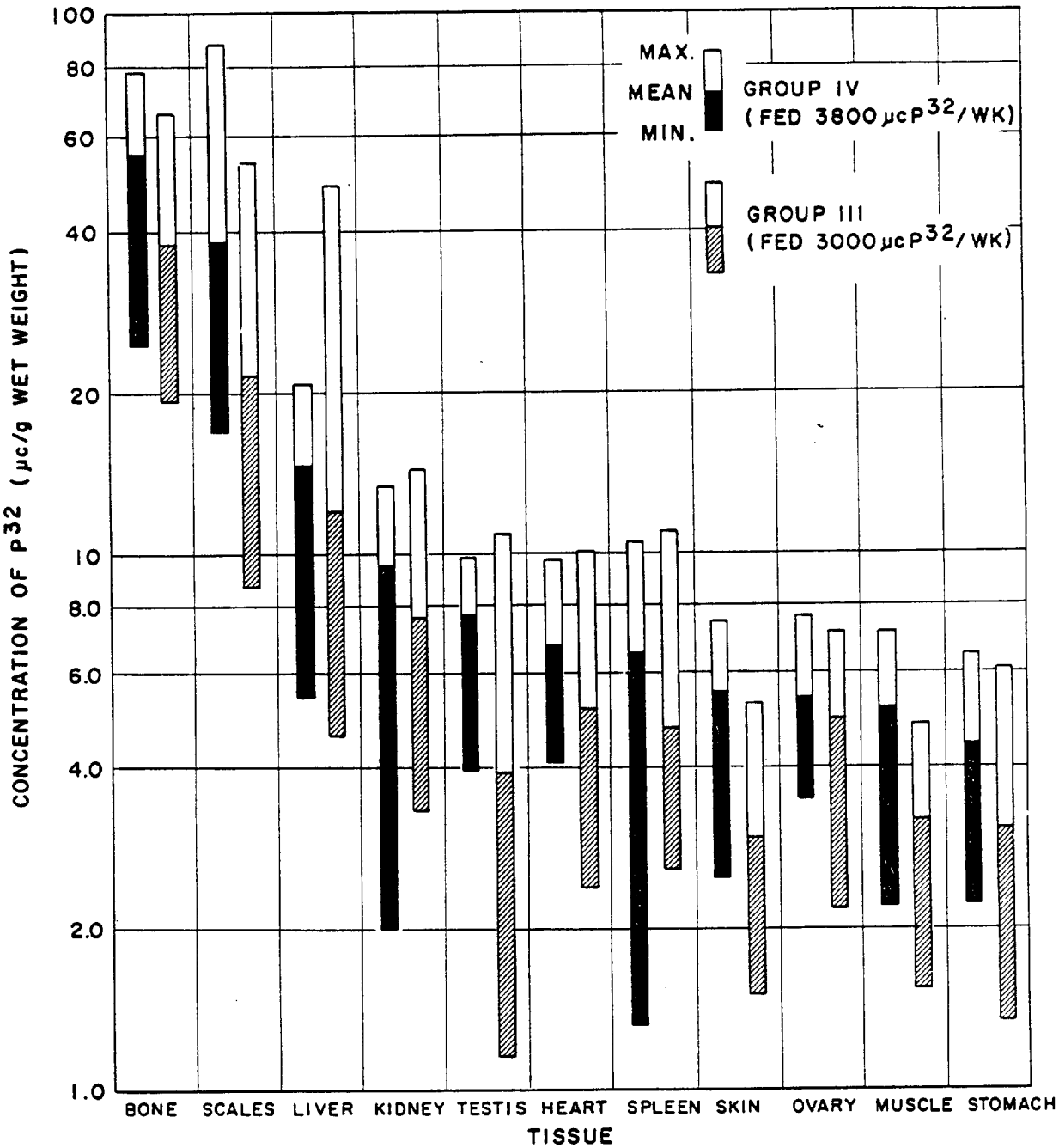

FIGURE 1
Concentration of P^{32} in Trout Tissues at Death

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To measure biological elimination of P^{32}, two fish from the low level group were killed and analyzed for P^{32} each week subsequent to the termination of the feeding of P^{32}. The uptake and loss of P^{32} in this group is given in Figure 2. The loss of phosphorus from bone was very slight, while the liver showed an appreciable reduction in P^{32}. The initial loss from muscle appeared to be slower than the radioactive decay of P^{32}. This suggests a continued uptake of the isotope by the muscle by translocation from other organs.

The results of this test show that P^{32} consumed at the rate of three millicuries per week by 200-gram trout is fatal within seven weeks. At death the mean concentration of P^{32} in bone, the tissue of maximum uptake, was 35 microcuries per gram wet weight for fish which received three millicuries each week. Fish fed 600 microcuries of radiophosphorus per week had a mean concentration of 10 microcuries per gram of bone at the end of 12 weeks. There was no mortality in this group but radiation damage was extensive and probably would have caused premature death. There was no evidence that bone was the critical organ. The gut and circulatory system showed much greater gross damage than bone.

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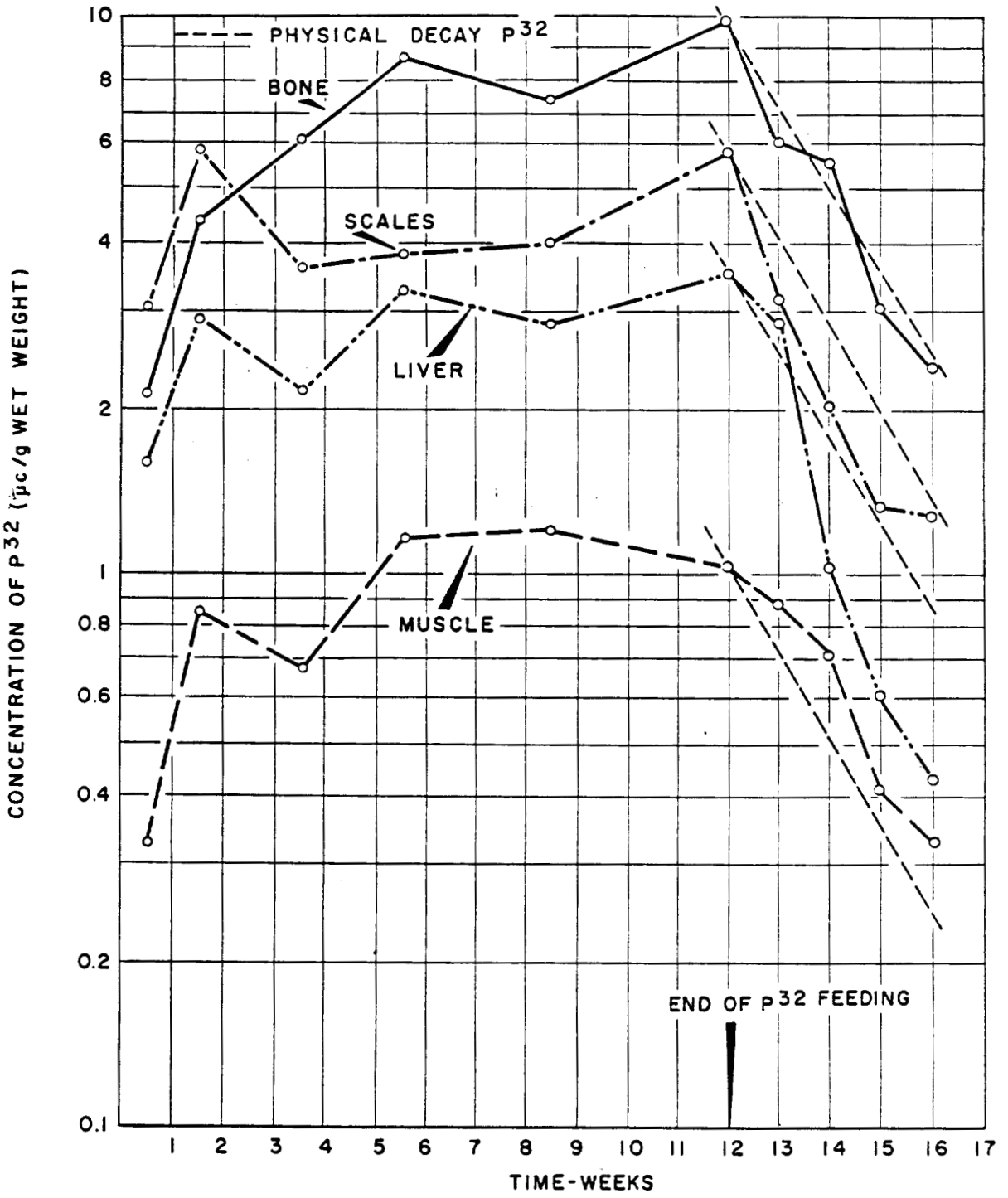

FIGURE 2

Uptake and Loss of P^{32} in Tissues of Trout Fed 600 µc P^{32} per Week.

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