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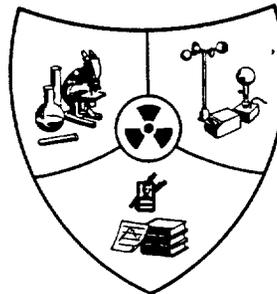
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1953 ANNUAL REPORT - BIOLOGY SECTION

INTRODUCTION

Previous volumes in this series that summarize the section's research activities for the calendar years 1951 and 1952 are HW-25021 and HW-28636. Papers comprising the major portion of this volume are of two types: progress reports of problems under investigations and abstracts of reports of completed problems that were published during the year.

To the heads of the units represented by papers presented - L. K. Bustad, R. F. Foster, F. P. Hungate, R. C. Thompson, and D. E. Warner - appreciation is expressed. To A. C. Case and personnel of the Radiochemistry Laboratory who provided the analytical services required, authors acknowledge gratitude. Assistance in editing this report was rendered by Dr. Thompson, and Evelyn G. Swezea coordinated preparation of the final draft.

H. A. Kornberg

Progress Report

1953 RADIOBIOLOGICAL SURVEY OF THE COLUMBIA RIVER

J. J. Davis, D. G. Watson, C. C. Palmiter and R. W. Coopey

Radiobiological conditions in the Columbia River during 1953 were comparable to those of previous years but with slight differences in radioactivity densities. Plankton had the highest activity density among organisms sampled between manufacturing areas and Richland, but small fish and caddis fly larvae were higher downstream from McNary Dam. This condition was attributed, in part, to the proportionally higher initial content of short-lived isotopes in plankton. Comparisons were made of abundance and radioactivity density of net and nannoplankton. During the winter and spring months relatively high radioactivity densities were found in some whitefish which had migrated upstream from the vicinity of reactor areas. A lower than average number of salmon nests within the environs of the Hanford project was consistent with small fall runs to other sections of the Columbia.

The general scope of the radiobiological survey which is being made of the Columbia River has been described in previous reports (1, 2). During 1953 representative forms of aquatic plant and animal life were again routinely sampled and analyzed for radioactivity density. Radiological and ecological studies of the McNary pool were continued. To better determine the geographical distribution of activity density in aquatic organisms, a short-term survey was made of the section of the river extending from Hanford to the mouth. The abundance and radioactivity density of net and nannoplankton from the Hanford area were compared. Since relatively high activity densities have been found in some whitefish caught upstream from the vicinity of reactor areas (2), sampling of these fish was intensified to evaluate possible radiation hazards and to study related biological aspects.

METHODS

Sampling methods for fish, bottom dwelling invertebrates, net plankton and algae were the same as previously described (1). To obtain nanoplankton, river water which had been filtered through No. 20 gauge silk bolting nets to remove net plankton was passed through a Foerst centrifuge operated at 20,000 rpm.

For radioactivity density analysis, plankton samples were filtered to remove excess moisture and the residue dried under infra-red lamps. Other biological samples were digested in concentrated nitric acid and ashed in a muffle furnace to reduce the mass. Water samples were treated with nitric acid and dried by boiling. Samples were counted in mica-window beta counters and the results expressed as $\mu\text{c/g}$ of live weight.

RESULTS AND DISCUSSION

Aquatic organisms sampled from the Columbia River within the environs of the Hanford project showed the same general radiological and biological conditions as reported for the preceding two years (1, 2). The seasonal and intraspecific patterns of activity density were unchanged. Slight differences in activity densities, as compared to values for the previous year, were related to corresponding changes in hydrographic conditions and manufacturing operations. Highest activity densities for most forms again occurred in the vicinity of Hanford.

The geographical distributions of activity density in two types of river organisms, plankton and small fish*, are shown in Figure 1.

*Small fish activity was determined from the compilation of the activity densities of various species of fish by use of intraspecific ratios which have been established from statistical comparisons.

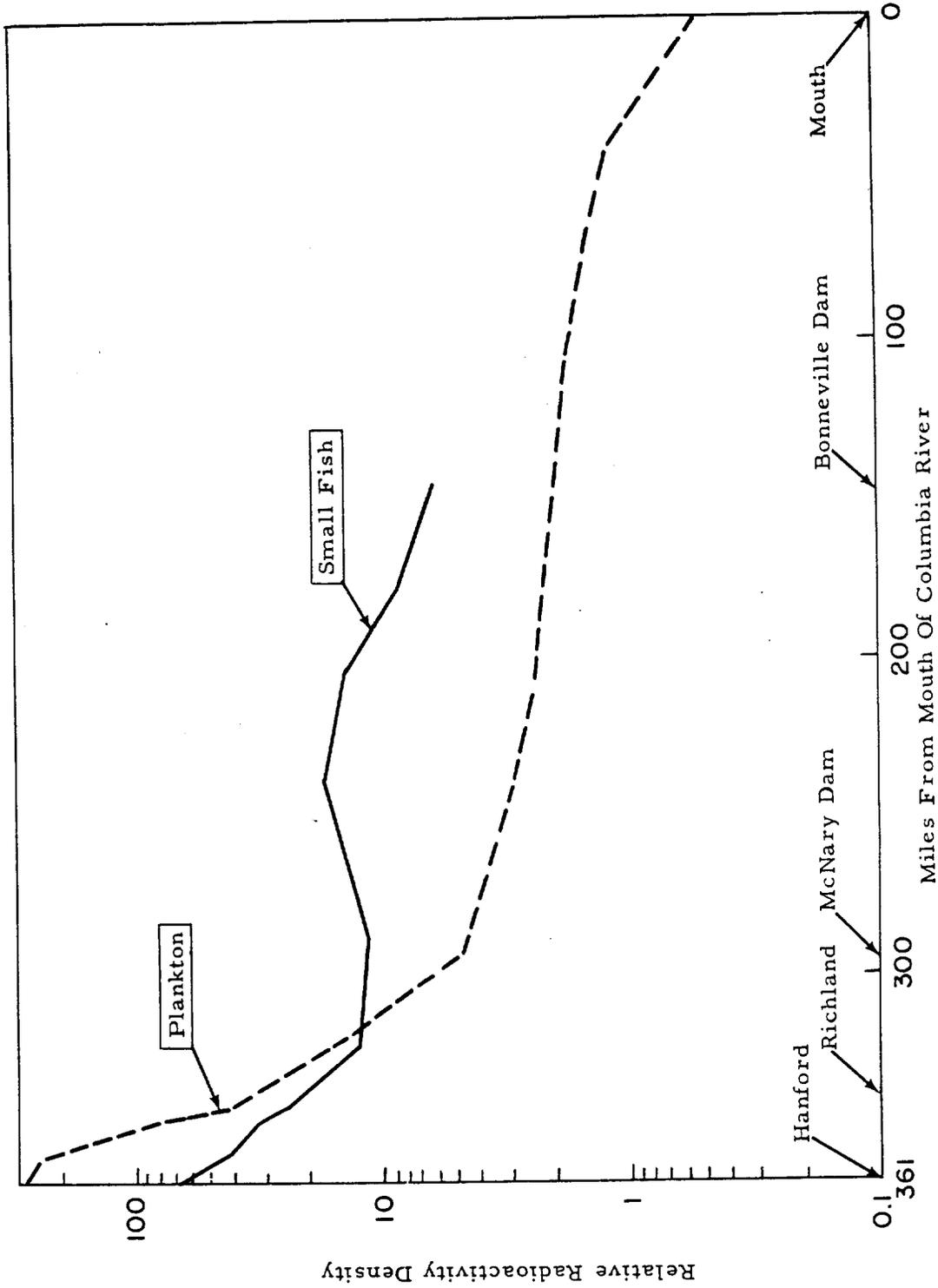


FIGURE 1
GEOGRAPHICAL DISTRIBUTION OF RADIOACTIVITY IN COLUMBIA RIVER ORGANISMS.

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Reduction of radioactivity in the river downstream from Hanford is attributed to radioactive decay, dispersion, sedimentation, assimilation by organisms and dilution. The initial rapid decrease in radioactivity downstream from Hanford is ascribed primarily to radioactive decay and dispersion. Among biological samples routinely collected between manufacturing areas and Richland, plankton has consistently been found to have the highest activity density. This condition apparently does not exist in the lower reaches of the river. Figure 1 shows that progressing downstream the activity of plankton decreases at a more rapid rate than that of other forms until down-river from the region of McNary Dam fish are of considerably higher radioactivity density than the plankton. Juvenile fish of two species - the shiner, Richardsonius balteatus, and squawfish, Ptychocheilus oregonensis - and larvae of the caddis fly, Hydropsyche cockerelli, all followed the same general pattern as shown for fish. The more rapid decrease in activity density of plankton is due, at least in part, to its proportionally higher content of short-lived isotopes. Additional sampling will indicate whether there is any seasonal variation in this relationship.

Comparison of nannoplankton and net plankton samples collected in the vicinity of Hanford indicated that for both fractions, maximum radioactivity densities of plankton occurred during the winter months when the activity density of river water was at a maximum. However, maximum radioactivity of the plankton contained in a unit volume of river water occurred during the late spring and summer when the abundance of plankton was at a maximum. By comparison of average values of samples collected throughout a year, nannoplankton exceeded the net plankton in radioactivity density by a factor of 16 and in dry organic weight by a factor of 8. Numerically, nannoplankters averaged 500 per ml of river water and net plankters 60 per ml. Radioactivity decay curves were similar, indicating a probable similarity in radioisotopic composition.

Approximately half of the whitefish caught during November and December of 1952 from the Priest Rapids area, located upstream from the Hanford reactors, showed activity densities equal to or greater than those of fish caught from sites immediately below reactor areas (2). High radioactivities continued to occur among fish caught from this area throughout the remainder of the low-water period, which was concluded in June by the spring freshet. The maximum activity densities found in whitefish from Priest Rapids during the first half of 1953 were 1.2×10^{-2} $\mu\text{c/g}$ of scales and 5.3×10^{-4} $\mu\text{c/g}$ of muscle in a specimen caught during May. Only background or very low levels of activity occurred in all fish sampled from this area during the summer months. High activities were again noted during October. This report was prepared prior to the corresponding period during which maximum activities were recorded last year.

Aerial observations of chinook salmon spawning showed a total of 149 salmon nests in the section of Columbia River between North Richland and Priest Rapids. While this is appreciably fewer than the 330 observed in the assumed parent year 1949, it is consistent with the poor fall run of chinook salmon to other sections of the Columbia River this year. The decrease is consequently not attributed to the Hanford reactors.

ACKNOWLEDGMENTS

We wish to acknowledge the technical assistance of R. G. Genoway, A. R. Williams, C. O'Malley and Patricia N. Hamilton.

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2. Davis, J. J., R. W. Coopey, D. G. Watson and C. C. Palmiter, "Radiological survey of the Columbia River," in "Biology Research - Annual Report 1952," Document HW-28636, p. 8 (1953) (UNCLASSIFIED).

Abstract

THE ABUNDANCE OF THE PRINCIPAL CRUSTACEA
OF THE COLUMBIA RIVER AND THE RADIOACTIVITY THEY CONTAIN

R. W. Coopey

The radioactivity and the abundance of crayfish and important small crustaceans of the Columbia River were determined for a 14-month period. In swift water habitats, exclusive of riffles, there was approximately one crayfish per square foot of bottom area; in eddies or riffles they were much less abundant. No significant reduction in numbers was apparent down-river from the reactors. Activity density of crayfish was highest from September through November and lowest from December through March. The activity density of the individual body organs, although showing similar seasonal trends, varied according to the metabolic rate of the particular organ concerned. The radioactivity in young, rapidly growing individuals was significantly higher than that of older specimens.

Smaller crustaceans in the bottom fauna were composed of nine species of Cladocera with a small component of Copepoda. A pronounced spring pulse and a smaller fall pulse were evident in the abundance of bottom Cladocera. Some 260,000 individuals per square foot weighing 2.6 grams occurred at the peak. No decrease was apparent downstream from the Hanford area. Activity densities were highest during the fall pulse with radioactivity of the immature forms exceeding that of the adults by one-third.

Both crayfish and Cladocera showed a marked drop in radioactivity levels downstream at Richland and McNary Dam with P^{32} as the principal isotope involved.

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A more detailed account of this investigation will be found in
HW-25191 (1).

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Progress Report

AN EXPERIMENT ON THE RELATION OF PHOSPHATE LEVEL IN WATER
TO THE REMOVAL AND CONCENTRATION OF RADIOPHOSPHORUS

R. H. Whittaker

Tracer P^{32} was introduced into aquarium communities with different levels of non-radioactive phosphate, ranging from 0.05 to 525 ppm. At higher phosphate levels (5 to 525 ppm) little of the P^{32} was removed from the water by organisms. At low phosphate levels (0.05 to 0.5 ppm) the P^{32} was rapidly and effectively removed and high concentration factors for P^{32} into the organisms resulted. The range between 0.5 and 5.0 ppm is thus the critical one separating effectiveness and ineffectiveness of biological removal of P^{32} contaminant. Limited prediction of removal and concentration effects for P^{32} on the basis of phosphate content of water is possible, but the relation is shown to be complex and non-linear.

Radiophosphorus has proved to be an isotope which is effectively concentrated by organisms in both the Columbia River (1) and White Oak Lake (2). The extent to which P^{32} is concentrated by organisms in aquatic environments depends, in part, on the level of natural phosphate in the water. It may not be possible to predict the extent of concentration from isotopic dilution alone, however; and the experiment to be described was designed to show effects of phosphate level on uptake and concentration of P^{32} . An experiment preliminary to the present study has been previously reported (3).

METHODS

Ten 60-gallon aquaria were filled with de-ionized water and fertilized with balanced inorganic nutrient solution, to a concentration one-twentieth that used here for Chlorella culture (4). Nutrient mixtures were the same in all aquaria except for varying concentrations of KH_2PO_4 ;

TABLE I
Distribution of Phosphates in Aquaria, as Related to Phosphate Concentration in the Water

Pair of Aquaria	1 and 2	3 and 4	5 and 6	7 and 8	9 and 10
a. Initial phosphate level	0.05	0.52	5.2	52.	520.
b. Per cent of non-radioactive phosphate remaining in water At tracer introduction 40 days later	35. 15.	5. 1.	75. 50.	99. 95.	100. 100.
c. Distribution of radiophosphorus in community fractions, 40-60 days, in per cent					
Water	2.1	1.7	53.	93.	99.
Filter plankton	1.4	.6	.4	.1	.04
Centrifuge plankton	2.4	.7	.6	.4	.04
Sidewall algae	2.7	2.4	1.5	.4	.2
Bottom algae	57.	50.	38.	4.3	.2
Sediment	5.7	6.7	3.1	.7	.3
Surfaces	29.	39.	3.7	.8	.1
d. Distribution of radiophosphorus in water + plankton, 5 days, in per cent					
Water	56.	86.	99.	99.	100.
Filter plankton	8.4	4.5	.6	.3	.1
Centrifuge plankton	36.	9.0	.4	.2	.05
e. Concentration factors, 5 days after tracer introduction					
Centrifuge plankton	3.6x10 ⁵	3.3x10 ⁴	2.5x10 ³	7.3x10 ²	2.5x10 ²
Sidewall algae	3.3x10 ⁴	8.4x10 ³	5.0x10 ²	2.2x10 ²	8.5x10
f. Per cent phosphorus content of algae, end of experiment	.21	.48	2.7	4.2	4.0

these formed a logarithmic series from 0.05 to 520 ppm (Table 1, a), with a pair of aquaria at each of the five phosphate levels. By replacing KH_2PO_4 with K_2SO_4 , the potassium content was kept constant in the aquaria with lower phosphate levels; the aquaria with highest phosphate content contained an excess of potassium as well as of phosphate. The lower phosphate levels represent normal magnitudes of phosphate levels in fresh-water bodies; Columbia River levels are of the same order as the 0.05 ppm aquaria but range generally below this level. Sampling procedures were essentially the same as those for the preceding experiment (3). Five weeks after the aquaria had been set up, $113 \mu\text{c}$ of P^{32} were introduced into each; and the samples were thereafter dried on plates and counted to determine content of P^{32} .

RESULTS AND DISCUSSION

The general pattern of removal of P^{32} from the water was similar to that in the preceding experiment (3). In the present experiment a larger portion of the P^{32} remained in the algae and a smaller part found its way to the sediment (Table 1, c) since no animals were present to convert attached algae into feces. Figure 1 illustrates the progress of removal of the P^{32} contaminant, as affected by initial phosphate level. In the aquaria of highest phosphate content (52-520 ppm), removal of P^{32} from the water was slight or insignificant. In aquaria with low phosphate levels (.05 and 5.0 ppm) removal of the P^{32} from the water was rapid and effective; within 2.5 days approximately 50 per cent had been removed, and after 60 days only 1 to 2 per cent remained in the water itself. Distribution of the P^{32} in the fluid portion of the aquarium system, the water plus plankton suspended in it, shows a further contrast (Table 1, d). At high phosphate levels only fractions of one per cent of the P^{32} could be found in the plankton; at low phosphate levels much of the P^{32} was concentrated in the rather minute organic mass of the plankton rather than in the water itself.

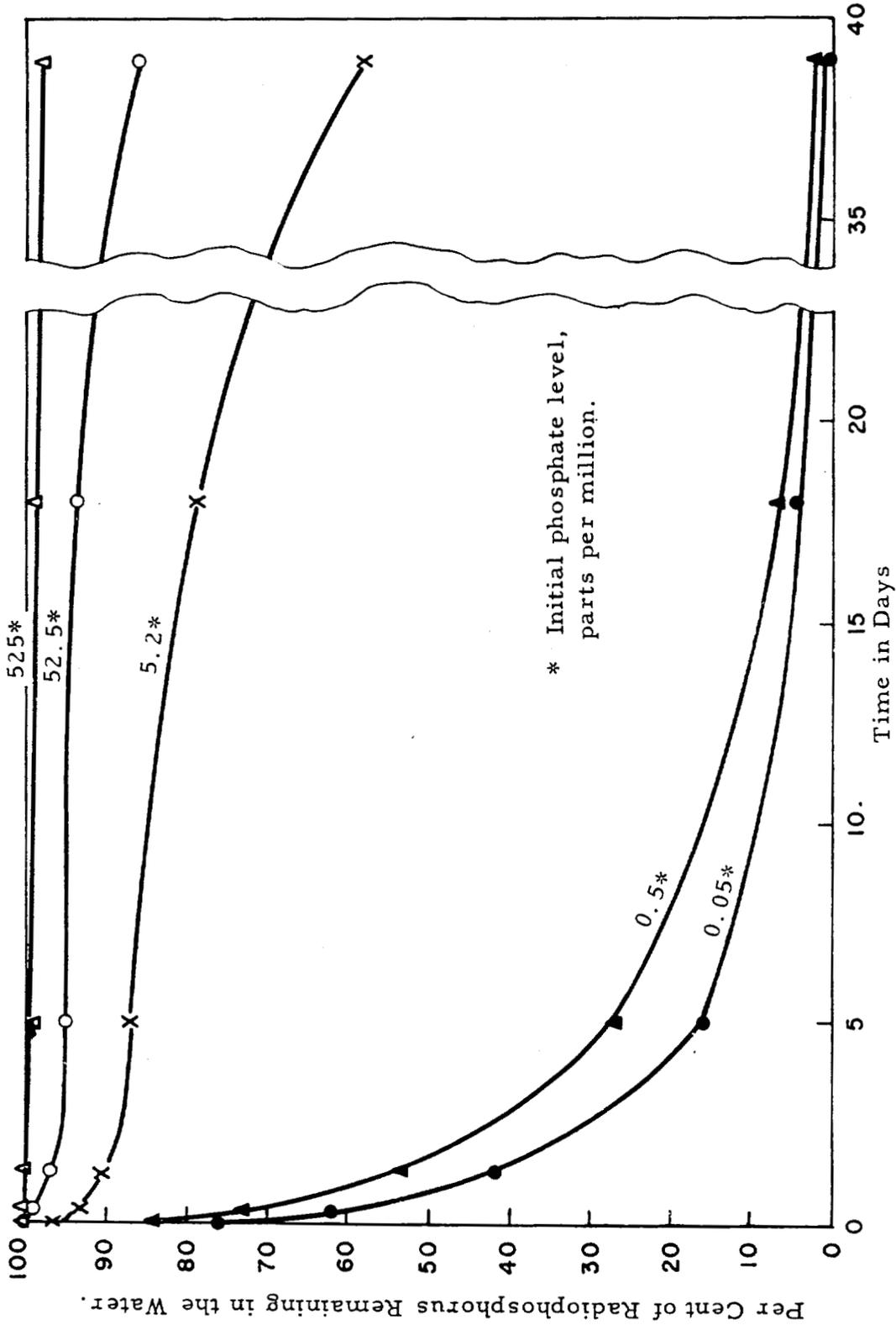


FIGURE 1
REMOVAL OF RADIOPHOSPHORUS FROM THE WATER OF AQUARIA, IN RELATION TO CONTENT OF NON-RADIOACTIVE PHOSPHATE.

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Concentration factors for P^{32} also were related to phosphate level (Table 1, e), and concentration factors for plankton were higher than those for attached sidewall algae. By the end of the experiment, indicated concentration factors had reached notable magnitudes in the low-phosphate aquaria: 9.5×10^5 for centrifuged plankton and 1.1×10^6 for bottom algae. A break or relatively steep change in the data is evident between 0.5 and 5.0 ppm. At the three higher phosphate levels P^{32} is only slowly removed from the water, but at the two lower levels it is rapidly removed and strongly concentrated into organisms.

In Figure 2 the expected relation of concentration factors to phosphate level, linear on a log-linear plot, is compared with the observed. Three factors are suggested as bases of the departure from expectation toward the sigmoid relation illustrated: 1. In the two low-phosphate levels the phosphate is removed from the water down to similar minimum levels (Table 1, b). Phosphate content and concentration factors in the four aquaria of low phosphate levels thus converged with time, flattening the upper slope of the curve in Figure 2. 2. In the high phosphate levels some of the original phosphate may have been in a form less available than the P^{32} introduced five weeks later, though still in the water. Some flattening of the lower slope of the curve may thus be accounted for. 3. In the middle range of the curve differences in phosphate content of algae (Table 1, f; cf 4) contribute to the departure from expectation. The "break" in concentration factors between the two sets of aquaria would be increased by a full order of magnitude if the algal cells contained equal amounts of phosphate, rather than one-tenth or one-twentieth as much at low phosphate levels.

As shown by these data and especially by Figure 1, the range between 0.5 and 5.0 ppm is the critical one separating effectiveness and ineffectiveness of biological removal of radiophosphorus contaminant. A difference of a few parts per million in this range may determine whether P^{32} introduced

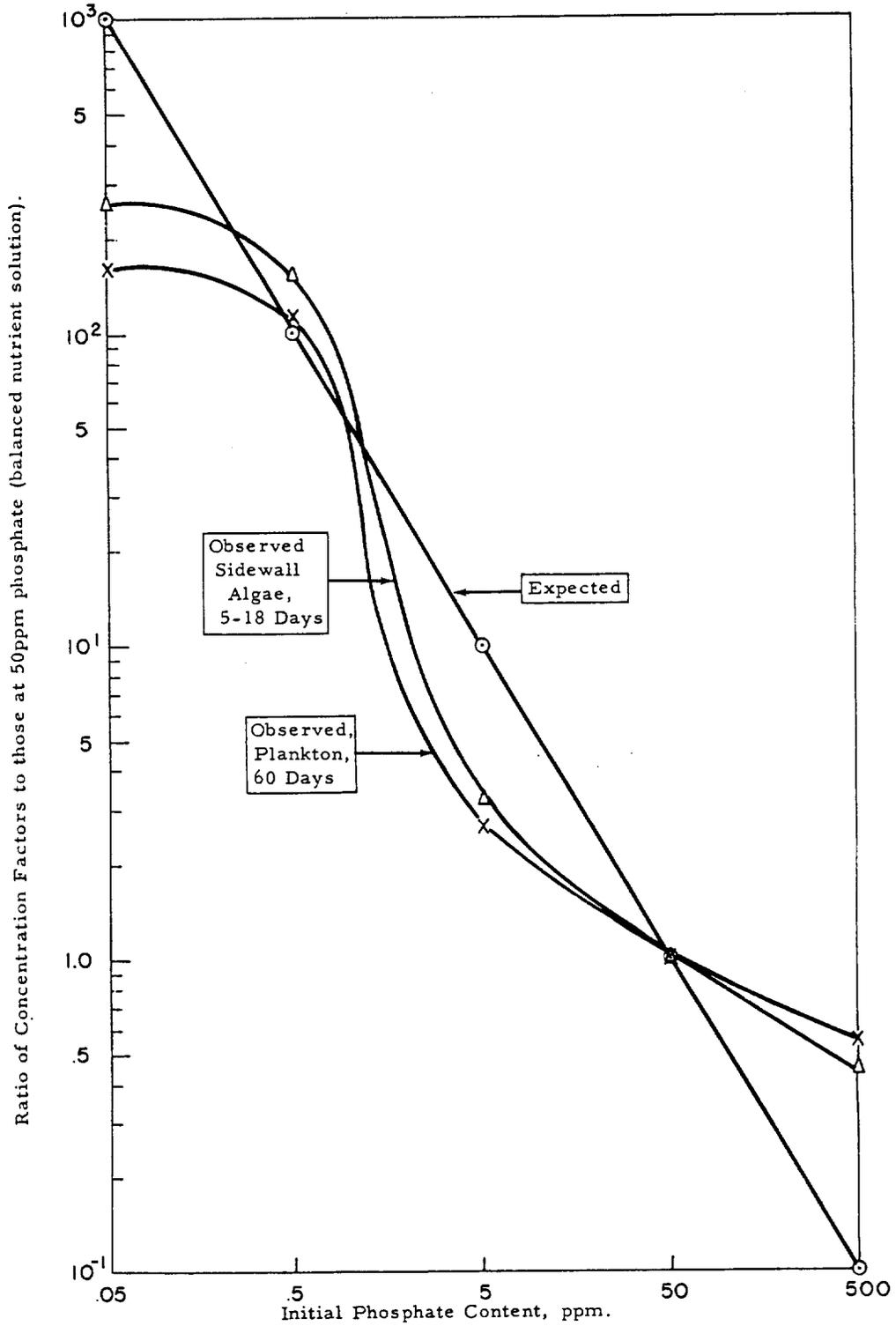


FIGURE 2
EXPECTED AND OBSERVED CONCENTRATION FACTORS OF RADIOPHOSPHORUS
INTO ALGAE IN RELATION TO PHOSPHATE LEVEL IN THE WATER.

into an aquatic community is largely concentrated into the tissues of organisms or remains largely dispersed in the water. Within this range concentration of P^{32} should be affected not only by phosphate level, but also by many other variables of aquatic communities. Departure of the results from simple expectation according to isotopic dilution may indicate the difficulties of prediction without regard to biological factors. In actual practice predictions often departed widely from observations in the low phosphate levels because of the inaccuracy of measurement of very small amounts of phosphate and of plankton, and because of the variation in phosphate content of the algae (Table 1, f).

The following conclusions in relation to radiophosphorus contamination problems are suggested:

1. Removal of P^{32} from water and concentration into organisms is strongly affected by isotopic dilution in natural phosphate, but the relation is a complex and non-linear one.
2. Knowledge of isotopic dilution gives some basis for prediction of concentration and removal effects; but, for various reasons, it seems difficult to make such prediction more than approximate.
3. Most natural aquatic environments are in the range of low phosphate levels in which effective removal from the water and strong concentration into organisms are to be expected (see 1, 2, 5, 6).
4. Fertilization of natural aquatic environments with non-radioactive phosphate might be considered as a means of reducing the concentration into organisms. Small and moderate amounts of phosphate tend, however, to be removed quickly from the water in natural aquatic environments (5, 6) as in the aquaria of this experiment.

ACKNOWLEDGMENTS

We wish to acknowledge assistance of R. E. Perey of Separations Technology, Analytical Laboratories, and W. H. Braymen of the Biology Control Unit in performing chemical determinations of phosphorous and technical assistance by C. O'Malley.

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Progress Report

REACTOR EFFLUENT MONITORING WITH YOUNG CHINOOK SALMON

P. A. Olson, Jr. and R. F. Foster

Chinook salmon eggs and young were subjected to a series of dilutions of reactor influent, dechlorinated reactor influent and reactor area effluent for a period of seven months. Survival of the eggs was slightly impaired in 2 1/2 per cent effluent, probably due to unfavorable temperatures. Significant mortalities resulted with reactor influent if residual chlorine was detectable, but dechlorinated influent was not toxic until chromate addition was resumed. No effect from radioactivity was observed since only low doses were received. Extrapolation to conditions existing in the river indicates no adverse effect on young salmon of the Columbia. Juvenile rainbow trout showed similar response, slight retardation of growth, but no increase in mortality occurred in 2 1/2 per cent area effluent and in 5 per cent reactor influent.

Earlier reports have described the objectives of this series of studies where young salmon or trout are subjected to dilutions of the reactor effluent water (1, 2, 3). Last year's report (3) covered a period ending in October, 1952; the work reported here describes results of the effluent monitoring with chinook salmon from November 1, 1952, through June 6, 1953, and with rainbow trout from June 12 through October 14, 1953. Subsequent studies will be reported next year.

CHINOOK SALMON

This study was designed 1) to determine the tolerance of young salmon to various dilutions of the effluent, 2) to compare the toxicity of the effluent with reactor influent water, and 3) to compare the toxicity of normal influent with that of influent with reduced chlorine content. A careful evaluation of the effect of the influent seemed appropriate at this time since chromate addition to the process water had been discontinued.

MATERIALS AND METHODS

On October 30, 1952, approximately 33,000 chinook salmon eggs, obtained from the Washington State Department of Fisheries hatchery at Soos Creek (Puget Sound drainage) were randomly distributed among twenty troughs with the following conditions:

Columbia River Water (3 troughs)

	1% Reactor Influent*		
2 1/2%	"	"	"
5%	"	"	"
10%	"	"	"
25%	"	"	"
50%	"	"	"
100%	"	"	"

	5% Dechlorinated Reactor Influent**		
10%	"	"	"
25%	"	"	"
50%	"	"	"
100%	"	"	"

	1% Reactor Area Effluent***		
2 1/2%	"	"	"
5%	"	"	"
10%	"	"	"
20%	"	"	"

The eggs and resulting fish were given normal fish-cultural care. Samples from each trough were weighed at two-week intervals and the lengths of 75 fish from each lot were taken at the end of the test. As the fish increased in size, the numbers held in each trough were reduced to

* Reactor influent is the purified river water supplied to the reactor as a coolant. In this study its temperature was adjusted to match that of the river water.

** Residual chlorine was removed by filtration through charcoal.

*** Reactor area effluent is a mixture of all industrial water discharged to the river but is almost entirely reactor coolant.

prevent overcrowding. The experiment was terminated on June 9 when the young fish were of sufficient size and age for migration to the ocean.

Shortly after the test was started, conditions beyond our control disrupted area effluent troughs for a period of about six weeks. Sodium dichromate, usually present in process water, was present only during the last eight weeks of the test. During the first two months of the test the residual chlorine content of the influent water at the laboratory was about 0.1 ppm. The level specified for process use was subsequently reduced and resultant residual at the laboratory was 0.05 ppm or less.

RESULTS AND DISCUSSION

Mortalities which occurred during the egg, fry and fingerling stages in the various concentrations of process water are shown in Figure 1. No significant mortalities occurred among any of the egg lots incubated in reactor influent water. A slightly greater mortality among eggs held in 100 per cent filtered influent is considered an experimental mischance, since it was not duplicated in unfiltered influent - a condition considered more severe. No significant mortalities occurred during the fry stage in the dechlorinated influent and fingerling mortalities in the 50 per cent and 100 per cent dechlorinated influent became abnormal only after dichromate addition was resumed. On the other hand, fry which hatched in the undiluted influent with a residual chlorine content of about 0.1 ppm died within a few days and those which hatched in 50 per cent influent survived less than one month. In 25 per cent unfiltered influent mortalities were about twice normal during both the fry and fingerling stages. Weaker mixtures did not affect survival.

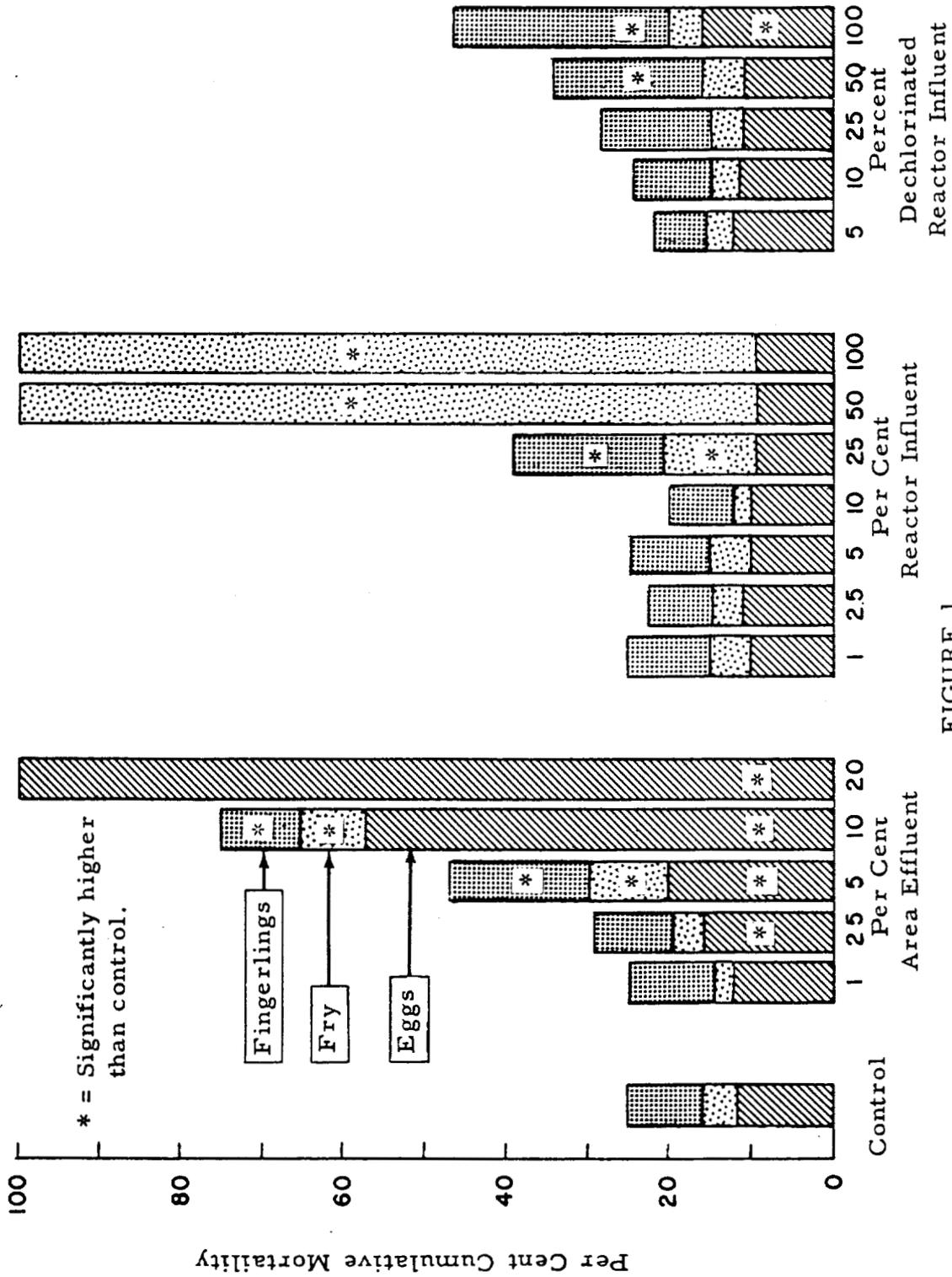


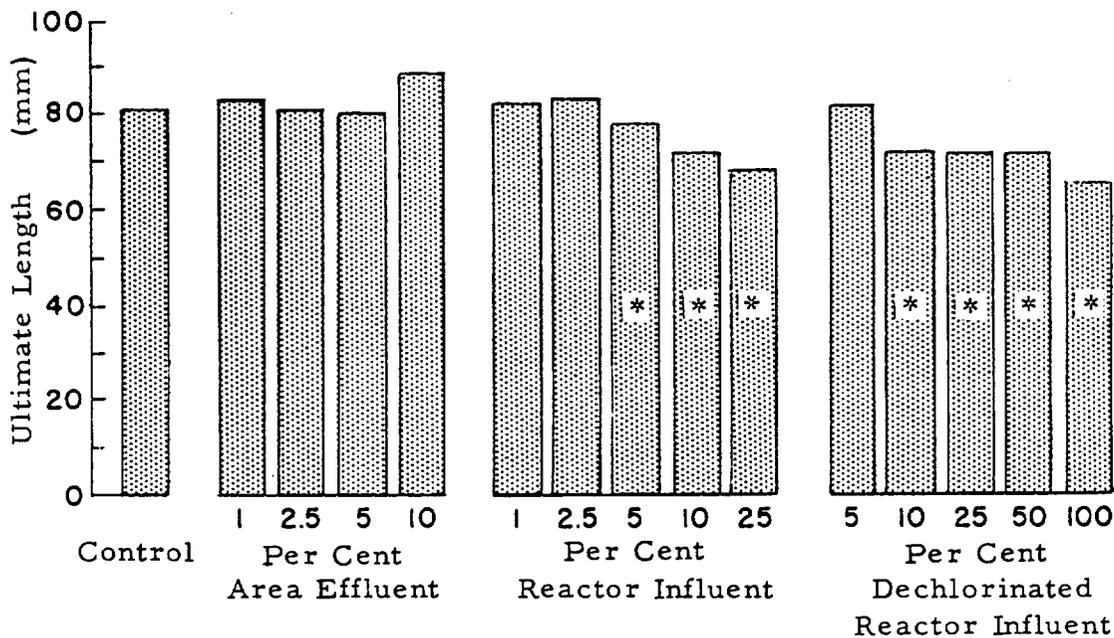
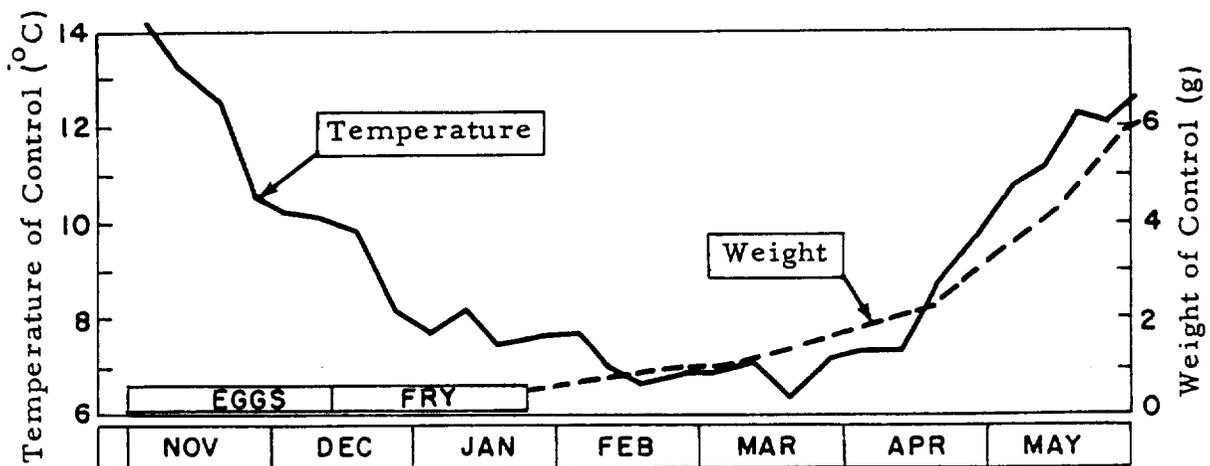
FIGURE 1
CUMULATIVE MORTALITIES OF CHINOOK SALMON IN VARIOUS CONCENTRATIONS OF REACTOR AREA WATERS.

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None of the eggs incubated in 20 per cent area effluent and only about half of those in 10 per cent area effluent survived. Abnormally high mortalities also were suffered by eggs held in 2 1/2 per cent and 5 per cent area effluent. During the fingerling and fry stages about twice as many fish died at the 5 per cent level as in the control troughs.

Figure 2 illustrates the growth pattern of the control fish and the ultimate size attained in the various conditions. In the untreated influent growth was slightly retarded in the 5 per cent level, while in the dechlorinated influent an adverse effect was first evident at the 10 per cent level. The higher temperatures of the area effluent mixture encouraged growth and this compensated for any retardation which might otherwise have been evident. The few surviving fish in the 10 per cent area effluent actually reached a larger size than the controls. Although analyses for activity density were made on the fish in some of the troughs containing the area effluent water, early results were variable due to changing conditions of the reactor effluent. When normal conditions prevailed, values were in good agreement with those obtained last year (3).

The results of this test would seem to indicate that survival of chinook salmon eggs was slightly impaired in 2 1/2 per cent reactor area effluent. Since the amount of radiation received by the embryos was insignificant, and since chemical toxicity was not otherwise manifested until the fry stage, mortality occurring during incubation of the eggs can probably be attributed to unfavorably high temperatures. The unseasonably high temperature of the Columbia River in the fall of 1952 was a contributing factor. It appears probable that Columbia River salmon can tolerate higher temperatures than the Puget Sound strain which were used in this test. While the eggs were apparently not affected by the chlorine in the water, the fry were susceptible to chlorine if present in more than trace amounts. Chlorine is probably not of great significance



* = Significantly smaller than control.

FIGURE 2

GROWTH OF CHINOOK SALMON IN VARIOUS CONCENTRATIONS OF REACTOR AREA WATERS.

in reactor effluent as discharged to the river, however, since heating of the water and subsequent retention should drive off most of the dissolved gas. Chromate toxicity became apparent in strong solutions of the influent when addition was again initiated late in the test.

Except for localized and well-defined areas immediately below the outfalls, concentrations of reactor effluent in the Columbia River ranged well below levels which, in the laboratory, produced adverse effects. It is doubtful that young salmon remained in localized areas of high effluent concentration for any appreciable period of time and, therefore, it seems reasonable to conclude that the Hanford reactors had no effect on the young salmon of the Columbia River during this period.

Additional data were obtained with one lot of young salmon which had been hatched and reared through the fry stage in Columbia River water of normal temperature. When this lot of fish had reached about 4 cm in length, the size of wild fish then abundant in the Columbia River, they were placed in 10 per cent area effluent. This lot tolerated the comparatively strong effluent, apparently without any adverse effect, for a period of 13 weeks until monitoring was concluded. Fingerling salmon originating in spawning areas upriver from the Hanford reactors could, therefore, pass through this section of the Columbia en route to the ocean without harm, even if effluent concentrations were many times those now encountered.

RAINBOW TROUT

Between the time that the chinook salmon fingerlings were released in June and the start of a new test with salmon eggs in October, monitoring of the reactor effluent was accomplished with rainbow trout obtained from the laboratory's brood stock.

METHODS

Experimental conditions set up for the salmon were utilized again for the trout. The young fish which were subjected to the different concentrations of area effluent, reactor influent and dechlorinated influent had recently hatched and were in the fry stage. They did not begin to feed until early in July, about one month after the test started. Records of mortality and growth were obtained in a manner similar to that for the salmon. In order to prevent serious epidemics of bacterial disease (Chondrococcus columnaris), sulfamerazine was routinely included in the diet at the rate of 25 milligrams per kilogram of fish during the months of August, September and October.

Reactor operation was essentially normal throughout the experiment and addition of chromate and chlorine to the process water was stable.

RESULTS AND DISCUSSION

The mortality pattern which resulted is shown in Figure 3. The effect of chromate toxicity was clearly evident since virtually no fish survived in troughs containing 100 per cent or 50 per cent reactor influent and appreciable mortality occurred at the 25 per cent level. Carbon filtration was of slight value in reducing mortality.

In 25 per cent area effluent, high temperature and chemical toxicity were intolerable and all of the trout died. Significant mortalities occurred at the 5 per cent level of area effluent but not at the 2 1/2 per cent level. (Uncontrolled disease caused an abnormal mortality in the trough containing 1 per cent effluent).

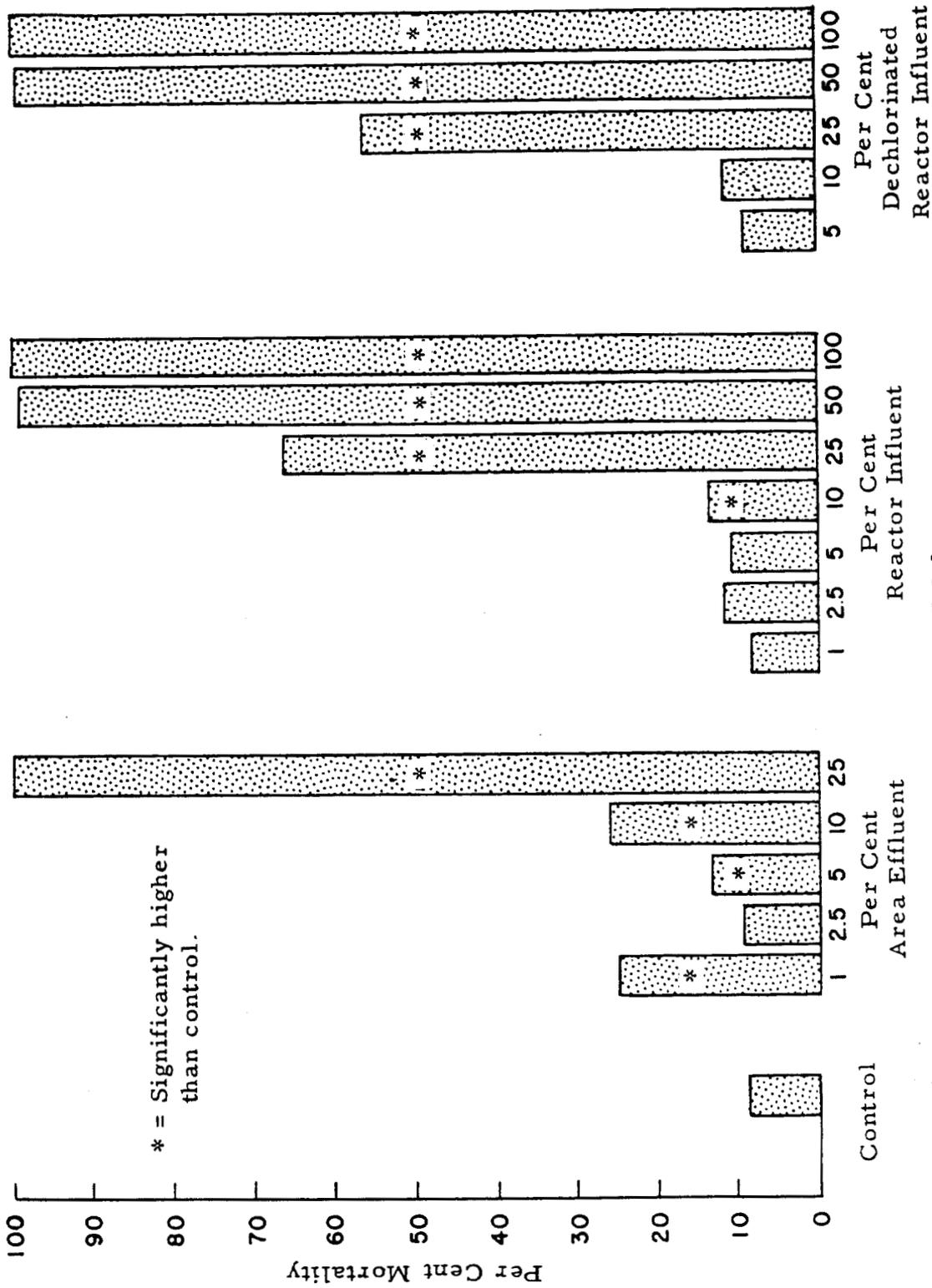


FIGURE 3
MORTALITIES OF RAINBOW TROUT IN VARIOUS CONCENTRATIONS OF REACTOR AREA WATERS.

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Rate of growth, illustrated by Figure 4, was a more sensitive index of chemical toxicity than mortality, and at the end of the study the fish in 5 per cent and greater concentrations of reactor influent and 10 per cent and greater concentrations of the dechlorinated influent were significantly smaller than the controls. The young trout also grew at a slower rate in 2 1/2 per cent and greater concentrations of the area effluent. Since growth is normally more rapid in warmer water as illustrated by the salmon, the retarded growth which occurred here indicates that temperatures were above optimum or possibly that an interrelationship exists between temperature and chemical toxicity. Effects resulting from the radioactivity are unlikely since, at the 5 per cent level of effluent, the fish received a dose of only about 5 rads over the entire experimental period.

ACKNOWLEDGMENTS

The assistance of Dr. L. R. Donaldson of the University of Washington in obtaining the salmon eggs and certain other supplies and his advice and counsel during the conduct of the test are gratefully acknowledged. Statistical analyses were made by the Control Unit of the Biophysics Section. Thanks are also extended to A. C. Schroeder and the Biological Attendants who assisted with the care of the animals, operation of equipment and recording of data.

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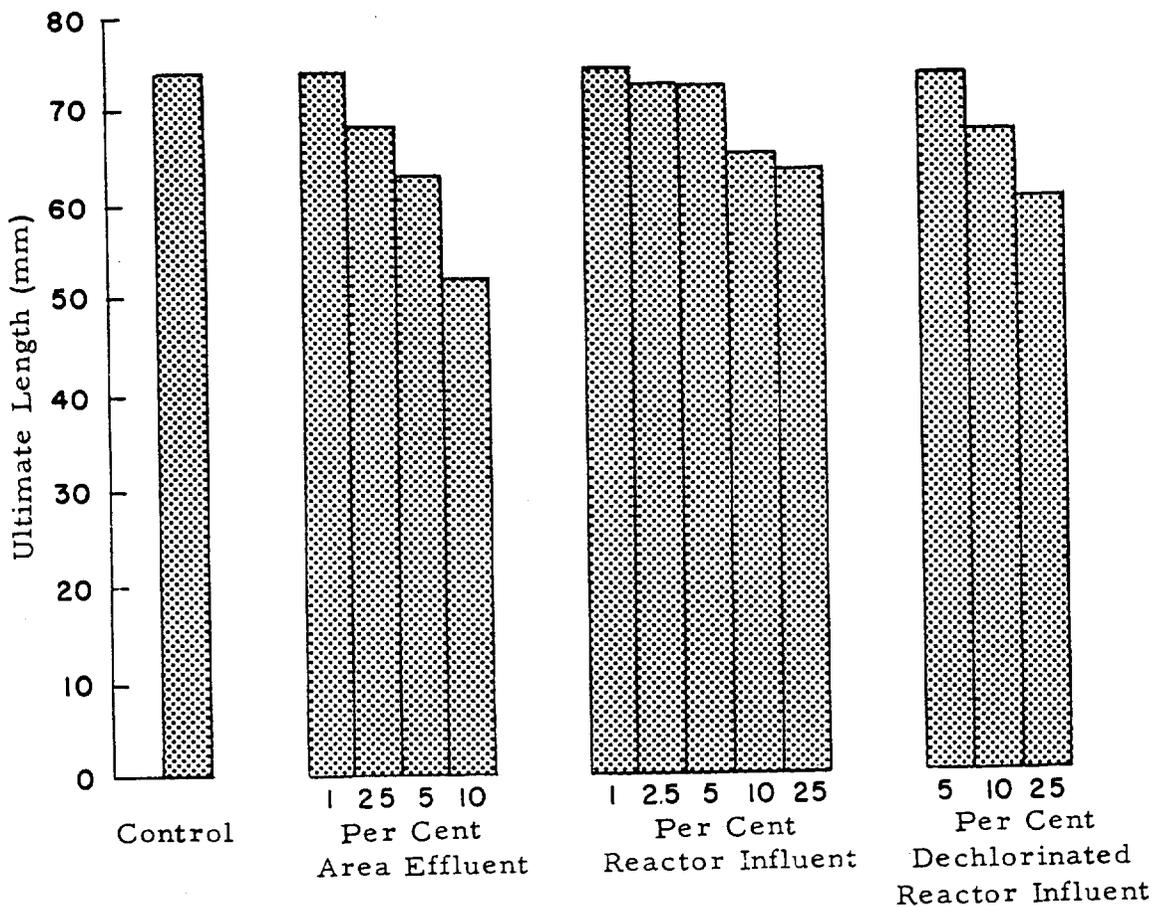
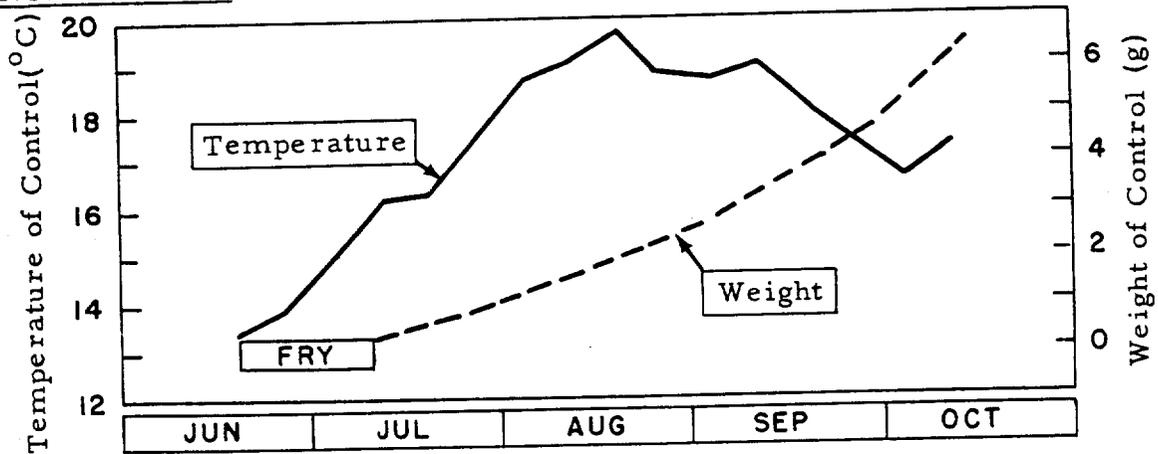


FIGURE 4

GROWTH OF RAINBOW TROUT IN VARIOUS CONCENTRATIONS OF REACTOR AREA WATERS.

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Progress Report

CHRONIC EFFECTS OF REACTOR EFFLUENT WATER ON
CEREAL PLANTS

J. F. Cline and J. H. Rediske

Milliacre plots planted to barley were irrigated with various dilutions of reactor effluent water in continuation of an experiment started in 1952. Effect of effluent on vegetation yield, seed yield, seed germination, and nitrogen concentration were evaluated. No effect was found in plots watered with five per cent effluent and only seed nitrogen was affected by 100 per cent effluent.

This experiment is the continuation of a chronic study to determine the effect of reactor cooling water on plants. The first year's results from this experiment were described in the previous annual report (1).

METHODS

Seed obtained from the previous year's crop was replanted in the same plot from which it was harvested. Watering, harvesting, and analyses were identical to those previously reported (1).

Plots were irrigated eight times during the season for a total water application of eleven milliacre inches. Activity densities were determined for each water at the time of irrigation.

A 48-liter sample of straight effluent water was filtered, and the solids and filtrate were submitted for spectrochemical analysis to quantitatively determine the elements present.

RESULTS AND DISCUSSION

Total yields of seeds and vegetative parts of the barley (Table 1) were higher than the previous year by a factor of approximately two. This may be largely attributed to a cooler growing season, which is conducive to growth of barley, and an improved soil structure resulting from settling and irrigation. Activity densities of the seeds were comparable to those of 1952, but the vegetation activity was slightly higher. The relative differences among treatments were the same for both years, with significant differences appearing only in the 100 per cent effluent treatment.

It appears that effluent water has an effect on seed nitrogen content, since the nitrogen concentration in the control seeds is greater than in those treated with 100 per cent effluent. Nitrogen per unit weight was less for all plots, compared with 1952 data. While the general decrease in nitrogen concentration of seeds in 1953 below that in 1952 is probably due to an increase in carbohydrate content, the lowering in the 100 per cent effluent plots probably indicates an impairment of protein synthesis.

The low activity density of seeds as compared to vegetation is what one might expect. Seeds consist primarily of carbohydrates and protein and contain only traces of those metals which constitute the major activity in reactor effluent. It therefore is expected that most of the radioactive elements will be deposited in the leaves rather than seeds.

Activity density of the soil and the germination of the seed are comparable to last year's data. Significant differences in yields of vegetation or grain were not observed. Lower yields obtained in the 100 per cent effluent plots the previous year (1) were probably due to the presence of chromate ion.

TABLE I

Summary of Results from Effluent-Watered Plots - 1953

	Control		5 Per Cent Reactor Effluent		100 Per Cent Reactor Effluent	
	Plot I	Plot II	Plot III	Plot IV	Plot V	Plot VI
Harvest Weight of Vegetation (g)	2920	2700	2330	2810	2830	3080
Harvest Weight of Grain (g)	1285	1295	1110	1280	1225	1150
Activity Densities of Vegetation ($\mu\text{c/g}$)	6.0×10^{-5}	5.0×10^{-5}	5.6×10^{-5}	6.5×10^{-5}	12.0×10^{-5}	9.4×10^{-5}
Densities of Grain ($\mu\text{c/g}$)	1.6×10^{-5}	1.5×10^{-5}	1.7×10^{-5}	1.2×10^{-5}	2.5×10^{-5}	2.6×10^{-5}
Total Seed Nitrogen (mg N/g)	19	17	16	17	16	15
Activity Densities of Soil ($\mu\text{c/g}$)	1.5×10^{-5}	2.2×10^{-5}	2.6×10^{-5}	1.7×10^{-5}	2.1×10^{-5}	1.8×10^{-5}
Germination (Per cent Viable)	99	99	100	100	100	100

The spectrochemical analysis of the effluent water showed aluminum, calcium, chromium, iron, magnesium, sodium and zinc present in concentration exceeding that considered desirable in good irrigation water. These may cause damage to the plants in later years as the elements accumulate in soil, since normal drainage is not possible due to a concrete pad placed about three feet below the surface of the soil.

ACKNOWLEDGMENTS

We wish to acknowledge the assistance of Analytical Laboratories, Technical Section in performing the spectrochemical analyses.

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Progress Report

THE ABSORPTION AND TRANSLOCATION OF Pu²³⁹ AND Ce¹⁴⁴
BY PLANTS

J. H. Rediske and A. A. Selders

The absorption of Pu²³⁹ and Ce¹⁴⁴ from a nutrient solution by four types of plants was studied as a function of concentration and pH of the nutrient environment. These elements exhibit a very low order of uptake efficiency, about 10^{-4} to 10^{-5} , which is at least three or four orders of magnitude less than for strontium.

Data from nutrient culture and soil experiments were previously reported for the uptake of the four fission elements: strontium, yttrium, cesium and iodine (1, 2). Also reported were data from nutrient culture experiments on a fifth element, ruthenium (2). Studies on plutonium and cerium are reported herein.

METHODS

Red Kidney beans were used as the principal experimental plant with supplemental studies being conducted on Rutgers tomatoes, Russian thistle, Victory oats and Sacramento barley. The culture of these plants was standardized (1) using a Hoagland solution. The concentration of the Pu²³⁹ was 50 d/m/ml of nutrient solution in the (IV) oxidation state except where otherwise indicated. The Ce¹⁴⁴ was used as a tracer at a concentration of 3.0×10^{-3} $\mu\text{c/ml}$ of nutrient solution with 1.0 $\mu\text{g/ml}$ of stable cerium as Ce(NO₃)₃ except in that experiment where concentration was a variable. The pH of the solutions was adjusted daily to 6.0 except in those experiments where pH was a variable.

RESULTS AND DISCUSSION

Plutonium

Figure 1 indicates that plutonium deposition on the roots of plants grown in nutrient culture is approximately a linear response to concentration. The uptake into the aerial portions, however, appears to increase at the higher concentrations (>250 d/m/ml of nutrient). If the leaf-root ratios are used as a measure of uptake efficiency (1), there appears to be a significant increase in uptake efficiency at 500 d/m/ml. Further experiments are needed to precisely outline the nature of the curve at these higher concentrations.

The concentration of plutonium associated with the roots (Figure 2) appears quite constant at pH values of 4 to 7. The uptake into the aerial portions, however, increases by about an order of magnitude as the acidity increases from pH 7 to 4.

There is little difference in the ability of those plant types tested to absorb plutonium, with the exception of the Russian thistle. This plant has an uptake efficiency about one order of magnitude less than the other three. All plants are characterized by a concentration of plutonium associated with the roots at least 4 or 5 orders of magnitude greater than in the aerial portions.

Cerium

The effect of stable cerium concentration on the uptake of Ce^{144} is presented in Figure 1. The amount of cerium associated with the roots as well as in the aerial portions increases uniformly with the nutrient concentration. The uptake efficiency, however, tends to decrease slightly at higher concentrations.

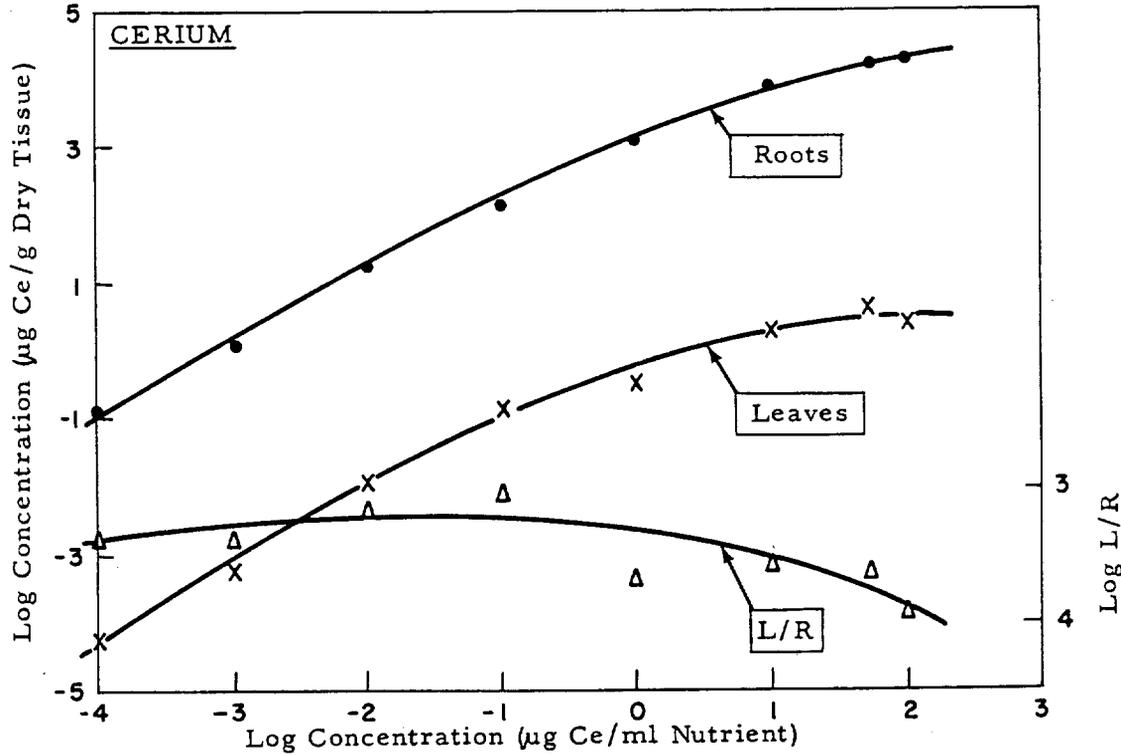
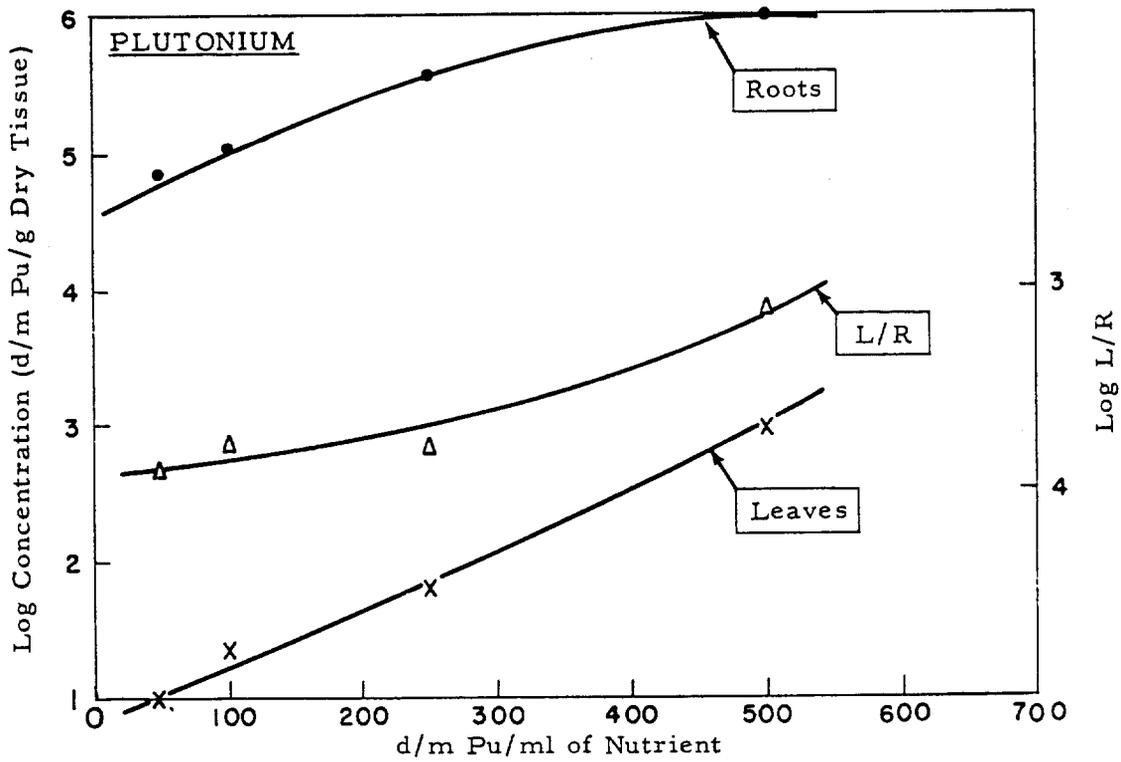


FIGURE 1
THE UPTAKE OF PLUTONIUM AND CERIUM BY BEAN PLANTS FROM A NUTRIENT SOLUTION AT A pH OF 6.0 AS INFLUENCED BY CONCENTRATION.

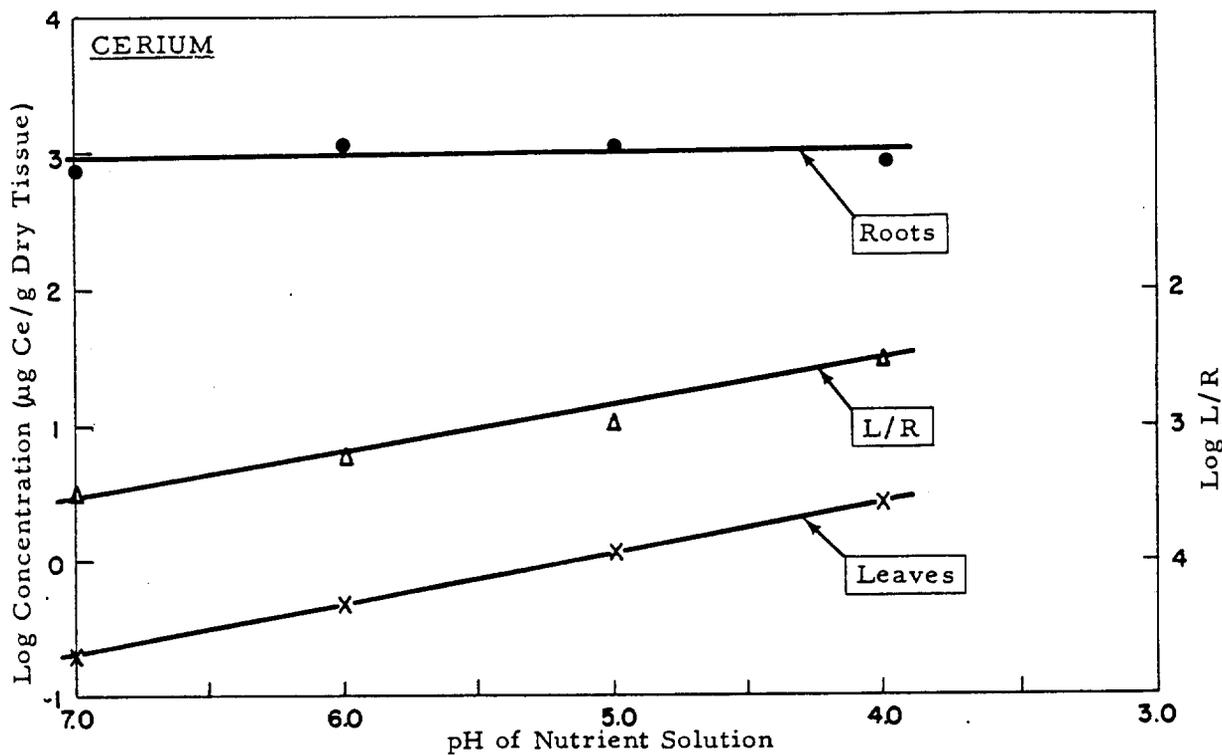
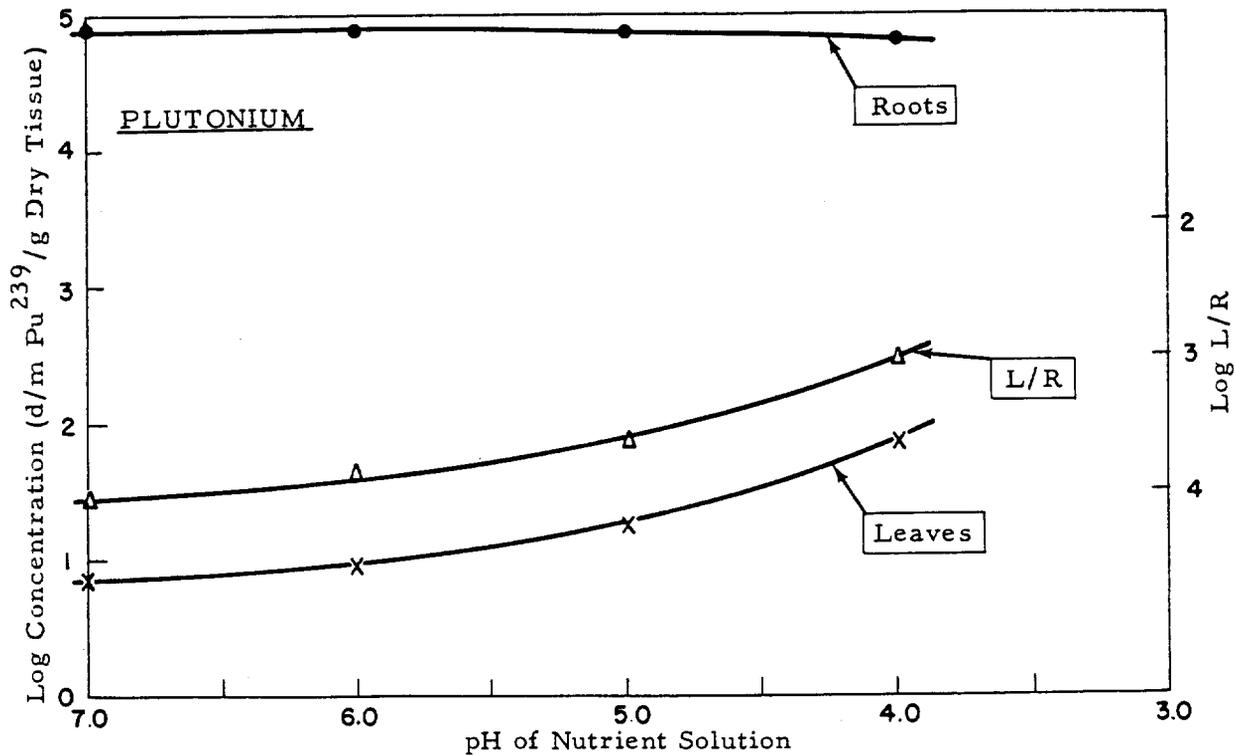


FIGURE 2
THE UPTAKE OF PLUTONIUM AND CERIUM BY BEAN PLANTS FROM A NUTRIENT SOLUTION AS INFLUENCED BY pH. THE CONCENTRATION OF CERIUM WAS 1 p.p.m. AND PU²³⁹ WAS 49 d/m/ml.

The effect of pH on cerium uptake (Figure 2) is not as pronounced as has been demonstrated for yttrium (1). The uptake efficiency increases approximately one order of magnitude over the pH range 7 to 4.

The uptake of cerium from a nutrient solution by the four plants studied is quite uniform. No pronounced differences in uptake efficiency are indicated (Figure 3).

ACKNOWLEDGMENTS

We wish to acknowledge the technical assistance of Josephine Sommers in this work.

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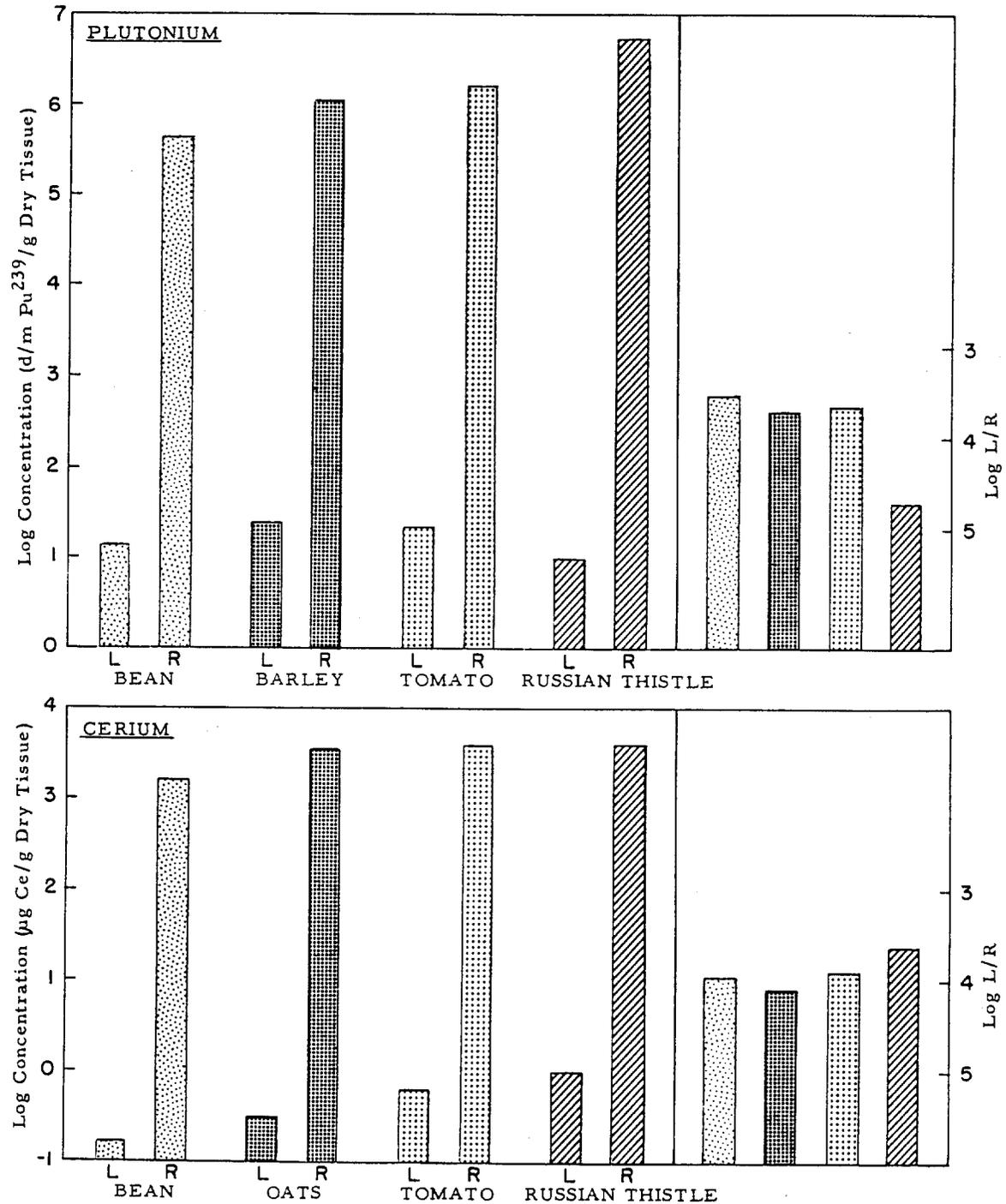


FIGURE 3
THE UPTAKE OF Pu^{239} AND CERIUM FROM A NUTRIENT SOLUTION BY FOUR DIFFERENT PLANTS AT A pH OF 6.0. EACH PLOT IS THE AVERAGE OF TWO CULTURES OF SIX PLANTS EACH. THE LEAF/ROOT RATIOS ARE PLOTTED ON THE RIGHT.

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Progress Report

PHYSICAL AND CHEMICAL PROPERTIES OF SEVERAL LOCAL
SOIL TYPES

J. F. Cline and J. H. Rediske

Nine local soil types to be used in comparative studies of fission product uptake by plants were collected and analyzed for particle size, soil pH, per cent organic matter, iodine content, and total nitrogen. The pH of the soils was neutral to slightly alkaline.

Nine soils were collected from the Hanford vicinity and the lower Columbia Basin area (1). The soil types, as designated and described by the U. S. Department of Agriculture (1), are Sagemoor sandy loam, Wheeler loam, Ritzville sandy loam, Quincy sand, Ephrata sand, Winchester sand, Ephrata fine sandy loam, Warden sandy loam, and Ringold clay loam.

The soil particle sizes were determined using the sedimentation method (2). Total nitrogen was determined using the micro-Kjeldahl apparatus. The iodine content was determined using a wet, alkaline (NaOH) digestion under pressure (3) followed by a ceric-arsenite colorimetric determination (4). The per cent organic matter was determined by the ignition method (5). The soil pH was determined using a wet paste method, measurements being made with a Beckman pH meter.

Soil particle sizes of the respective soil types are summarized in Table 1. With the exception of Ringold clay loam, all soils were of sandy texture which is characteristic of East Central Washington.

Table 2 summarizes iodine content, per cent organic matter, soil pH and total nitrogen of the nine soil types. All soil types were low in total nitrogen and per cent organic matter compared with an ideal soil.

TABLE 1
Distribution of Particle Sizes (Per Cent of Total Weight)

Soil Type	2-1 mm	1-0.5 mm	0.5-0.25 mm	0.25-0.1 mm	0.1-0.05 mm	0.05-0.002 mm	0.002 mm
Winchester sand	0.7	5.2	15.5	55.6	15.9	2.4	4.7
Quincy sand	1.0	9.0	20.3	37.8	21.3	6.4	4.2
Ephrata sand	2.4	3.2	6.4	33.6	29.2	19.0	6.2
Warden sandy loam	0.1	1.6	4.1	20.0	52.1	17.2	4.9
Ritzville sandy loam	0	0.5	2.2	23.0	45.4	24.1	4.8
Sagemoor sandy loam	1.6	1.2	4.9	25.2	39.7	21.7	5.7
Ephrata fine sandy loam	13.3	8.0	4.5	22.6	39.2	6.6	5.8
Wheeler loam	0.1	0.3	0.4	4.5	26.5	63.9	4.3
Ringold clay loam	0.4	0.9	3.0	7.4	7.3	40.4	40.6

TABLE 2
Chemical Characteristics

Soil Type	Total N mg N/g	Iodine Content $\mu\text{g/g}$	pH	Per Cent Organic Matter
Winchester sand	.35	.059	7.4	2.4
Quincy sand	0	.032	7.3	1.9
Ephrata sand	.19	.068	7.5	1.9
Warden sandy loam	.49	.048	7.7	2.7
Ritzville sandy loam	.42	.022	7.8	3.0
Sagemoor sandy loam	.16	.135	8.2	2.8
Ephrata fine sandy loam	.39	.073	7.3	1.8
Wheeler loam	.52	.058	7.7	4.3
Ringold clay loam	1.18	.072	7.6	7.9

The pH of the soils was neutral to slightly alkaline. The iodine content was determined to facilitate future experiments using I^{131} .

ACKNOWLEDGMENTS

We wish to acknowledge the assistance of Patricia L. Hackett of the Biology Control Unit who performed the iodine determinations, and D. W. Rhodes of the Biophysics Section who performed the soil particle size determinations.

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Progress Report

THE DESTRUCTION OF RIBOFLAVIN, BIOTIN AND FOLIC ACID IN
VERY DILUTE AQUEOUS SOLUTION BY TRITIUM BETA RADIATION

M. E. Getzendaner and M. P. Fujihara

A study was made of the radiosensitivity of dilute aqueous solutions of biotin, folic acid and riboflavin. The solutions, with a vitamin concentration of 10 $\mu\text{g}/\text{ml}$, were prepared from specially purified water, and were saturated with either oxygen or nitrogen. Tritium incorporated in the solution in the form of its oxide served as a source of radiation. Folic acid was found to be the most sensitive to radiation of the three tested. Radiation destruction of biotin was most sensitive to oxygen. This gas caused more rapid destruction by radiation of all three vitamins.

The sensitivity of the B vitamins to radiation has been studied to some extent previously. Dunlap and Robbins (1), while irradiating 0.1 μM solutions of thiamin chloride with X rays, P^{32} and radon, found a progressive depletion of activity of the solutions when sufficiently large doses were administered. Tchaperoff (2), using X rays, noted an exponential decrease in vitamin concentration with increasing dose when 10^{-4} μg of p-aminobenzoic acid were initially present per ml of solution. The p-aminobenzoic acid was inactivated by as little as 50 r with 190 KV X rays. Assays for remaining vitamin were carried out microbiologically in both cases. Mar and Tchaperoff (3) also found that $\text{m}\mu\text{M}$ solutions of p-aminobenzoic acid were 50 per cent inactivated by about 1600 r of 230 KVP X rays. In a series of papers initiated by Proctor and Goldblith (4, 5, 6, 7, 8, 9) the radiosensitivity of several metabolites including nicotinic acid, p-aminobenzoic acid, riboflavin and vitamin B_{12} was determined. The concentrations used in this work ranged from 5 to 100 $\mu\text{g}/\text{ml}$. It was demonstrated that the stability of the vitamins increased with increasing concentration. In all cases, doses of the order of 10^4 to 10^5 rep* were

*Although the currently accepted unit is the rad, this paper was prepared prior to notification of the change from rep to rad (1 rad = 1.08 rep).

required to show marked destruction. Vitamin B₁₂ at 10 µg/ml was the most sensitive, being half destroyed by 8.1×10^3 rep with 3 Mev cathode rays.

It appeared desirable to determine the radiosensitivity of some of the B vitamins in a more nearly physiological concentration. This was carried out with riboflavin, folic acid and biotin using the beta radiation from tritium incorporated in the solution as the oxide. The vitamin remaining in solution following irradiation was determined by microbiological assay. To demonstrate that the assay was specific for the vitamin itself, and was unaffected by any of its radiation products, samples of irradiated vitamin solution were subjected to two dimensional paper chromatography. The chromatograms were analyzed by the bioautographic technique (10).

EXPERIMENTAL

The organism used for biotin and folic acid assays was Lactobacillus casei ATCC 7469. The medium was that of Roberts and Snell (11) for vitamin assays. Riboflavin assays were carried out as described by Kornberg, et al (12) using Streptococcus zymogenes, ATCC 10100, which was tentatively called Leuconostoc mesenteroides P-60 by those authors.

Materials and Procedure

The riboflavin and folic acid used were obtained from General Biochemicals, Inc. and the biotin from Nutritional Biochemicals Corp. The water was triply distilled using utmost care to avoid contamination by organic material. This triply-distilled water was autoclaved and saturated with oxygen or specially deoxygenated nitrogen (13). Preparation of the tritium oxide solution was similar to that of the water except that the final distillation was made into a sterile flask and an oxygen or nitrogen atmosphere maintained during and following the distillation so that this

solution was also gas saturated. To maintain the desired atmosphere, all flasks were filled with the gas before addition of liquid. Air was excluded during transfer operations by flushing the flasks with the nitrogen or oxygen during the transfer.

Irradiations were carried out in glass stoppered "low actinic" pyrex flasks to prevent loss by the action of light. The radiation dosage was varied both by varying the time of exposure and by the addition of different concentrations of tritium oxide to the exposure flasks. In all cases the initial concentration of vitamin in the solutions for irradiation was 10 $\mu\text{g}/\text{ml}$.

Vitamin assays were made in 18 x 150 pyrex tubes, matched for use in the Coleman Spectrophotometer. At each dose a series of aliquots of irradiated vitamin solution was assayed to be sure that the vitamin concentration was within the range of the test organism. Determination of vitamin concentration was made by interpolation from a standard curve. The irradiated solution was examined bioautographically for the presence of radiation-produced compounds either inhibitory or stimulatory to growth. The procedure used was an extension of that described by Harrison (10) using two dimensional development of the paper chromatogram with butanol:pyridine:water one way and the upper phase from a mixture of butanol:acetic acid: and water the other way. After drying, to remove the solvents, the chromatograms were placed for 10 minutes on the surface of the medium in large plates. The medium in these plates was deficient in the vitamin being tested and contained bromcresol purple as an indicator. It was uniformly inoculated at the time the plates were poured. After 24 hours' incubation, the presence of as little as 0.025 μg of biotin or folic acid could be identified by the change in color of the indicator. The vitamins or other growth promoting materials induced growth which caused acid production and produced a localized color change from blue to yellow.

Tests for inhibitory substances were made by adding to the plates 0.1 μg of the vitamin per ml of medium. Inhibition appeared as intense blue spots on a pale blue background. Bioautographs of riboflavin were not made.

RESULTS AND DISCUSSION

The results of the destruction studies are shown in Figures 1, 2 and 3. It can be seen that the fraction of vitamin remaining in solution, f , decreases exponentially with dose, D , according to the equation

$$f = e^{-KD}$$

where K is the value of the slope of the curves. Table 1 lists the values of K for the three vitamins tested. It is of interest to note that riboflavin is twice as resistant to destruction in oxygen saturated solution as is either biotin or folic acid.

Biotin, which is nearly as sensitive as folic acid in oxygen saturated solution, is destroyed only a third as rapidly in oxygen free solution. The greatest difference in rate between oxygen and nitrogen saturated solutions is in the case of biotin.

Paper chromatographic analyses were carried out to determine whether any of the radiation products could either replace the vitamin in supporting growth, or cause inhibition of growth. If either effect were found, the destruction curves would require correction. Bioautographs of the chromatograms indicated that the only material supporting growth was located in the spot corresponding to the vitamin, and no cases of inhibition were detected.

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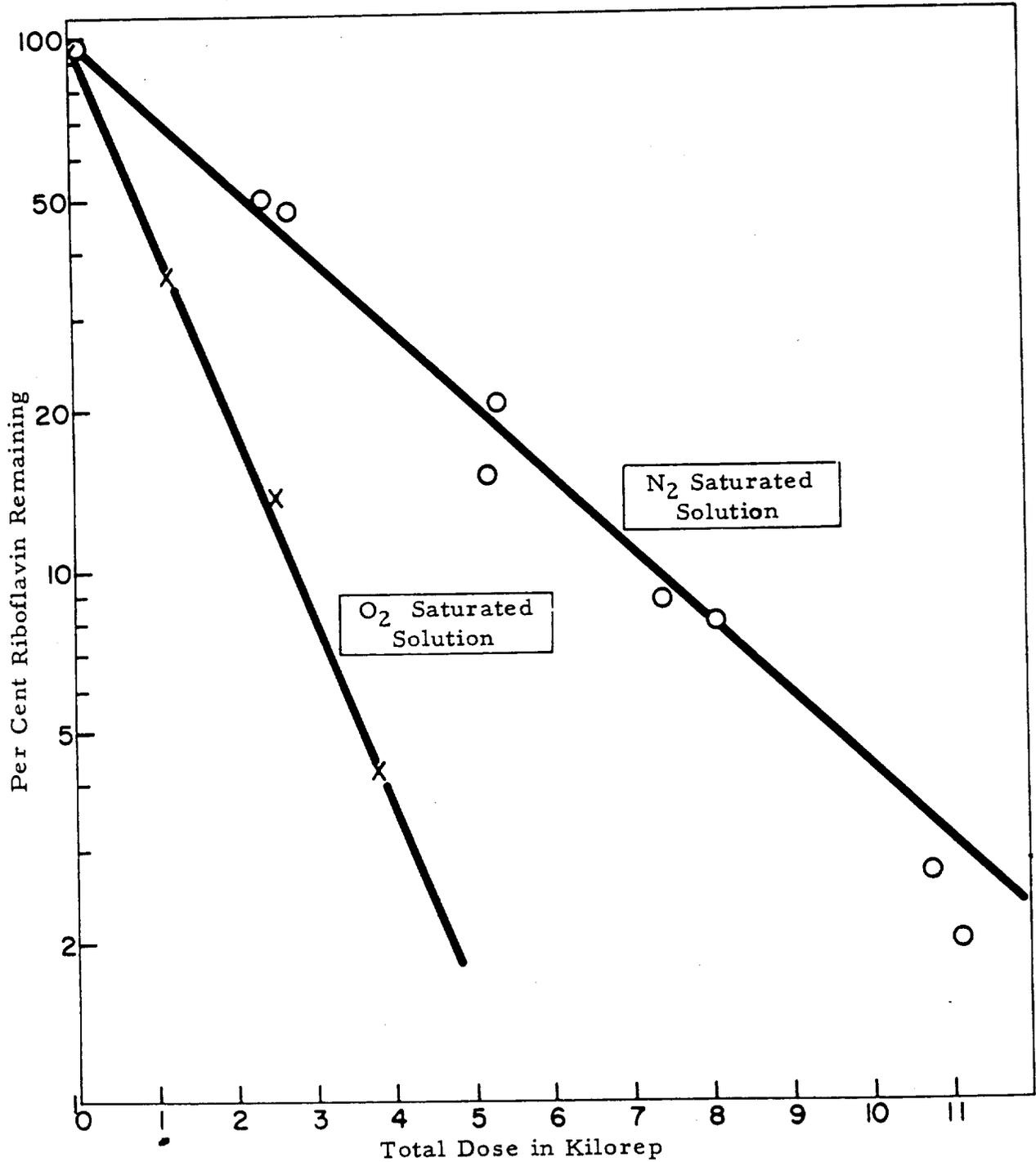


FIGURE 1
DESTRUCTION OF RIBOFLAVIN IN AQUEOUS SOLUTION BY TRITIUM
IRRADIATION. INITIAL RIBOFLAVIN CONCENTRATION 0.010 μg/ml.

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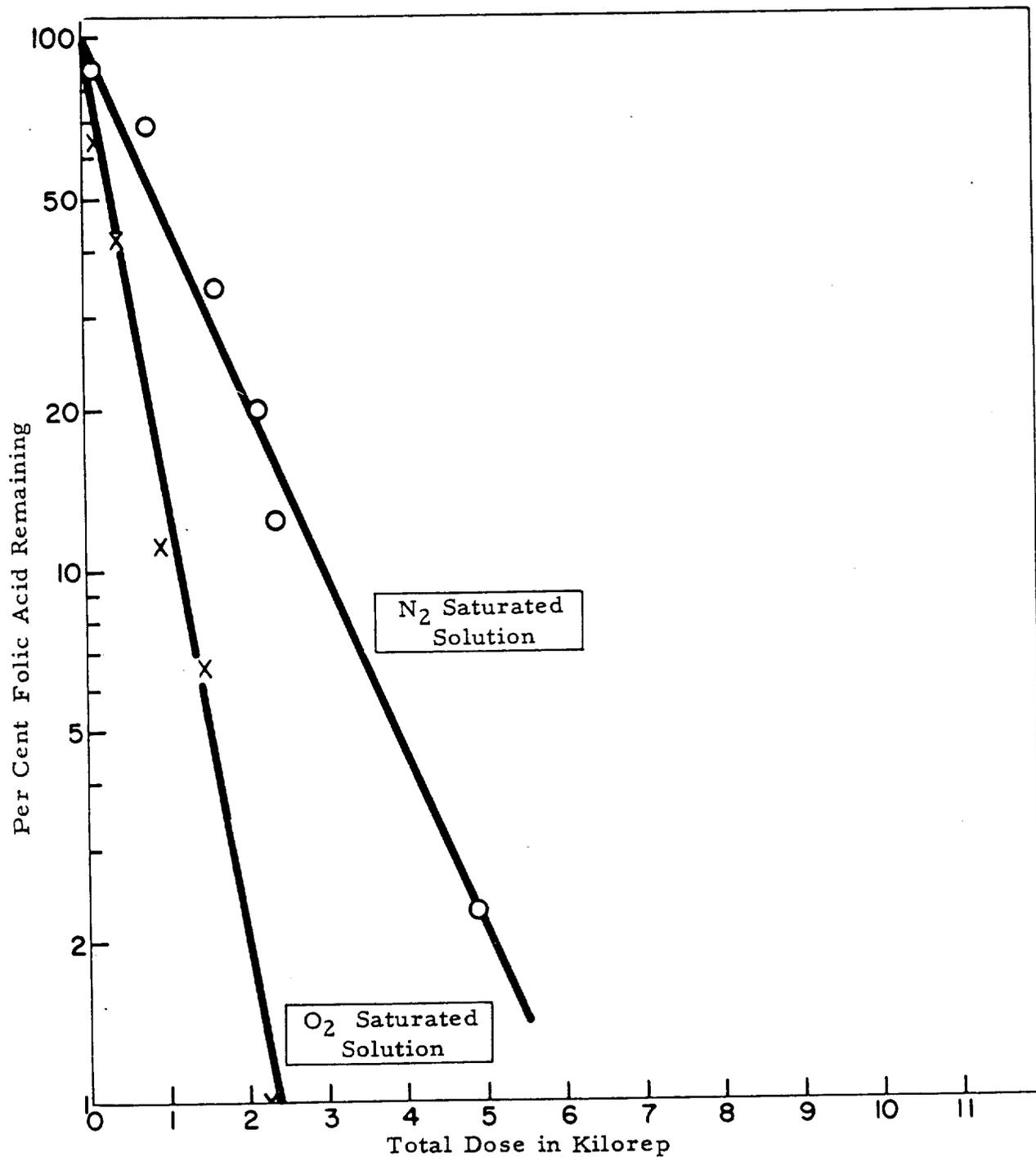


FIGURE 2
DESTRUCTION OF FOLIC ACID IN AQUEOUS SOLUTION BY TRITIUM
 β IRRADIATION. INITIAL FOLIC ACID CONCENTRATION 0.010 μ g/ml.

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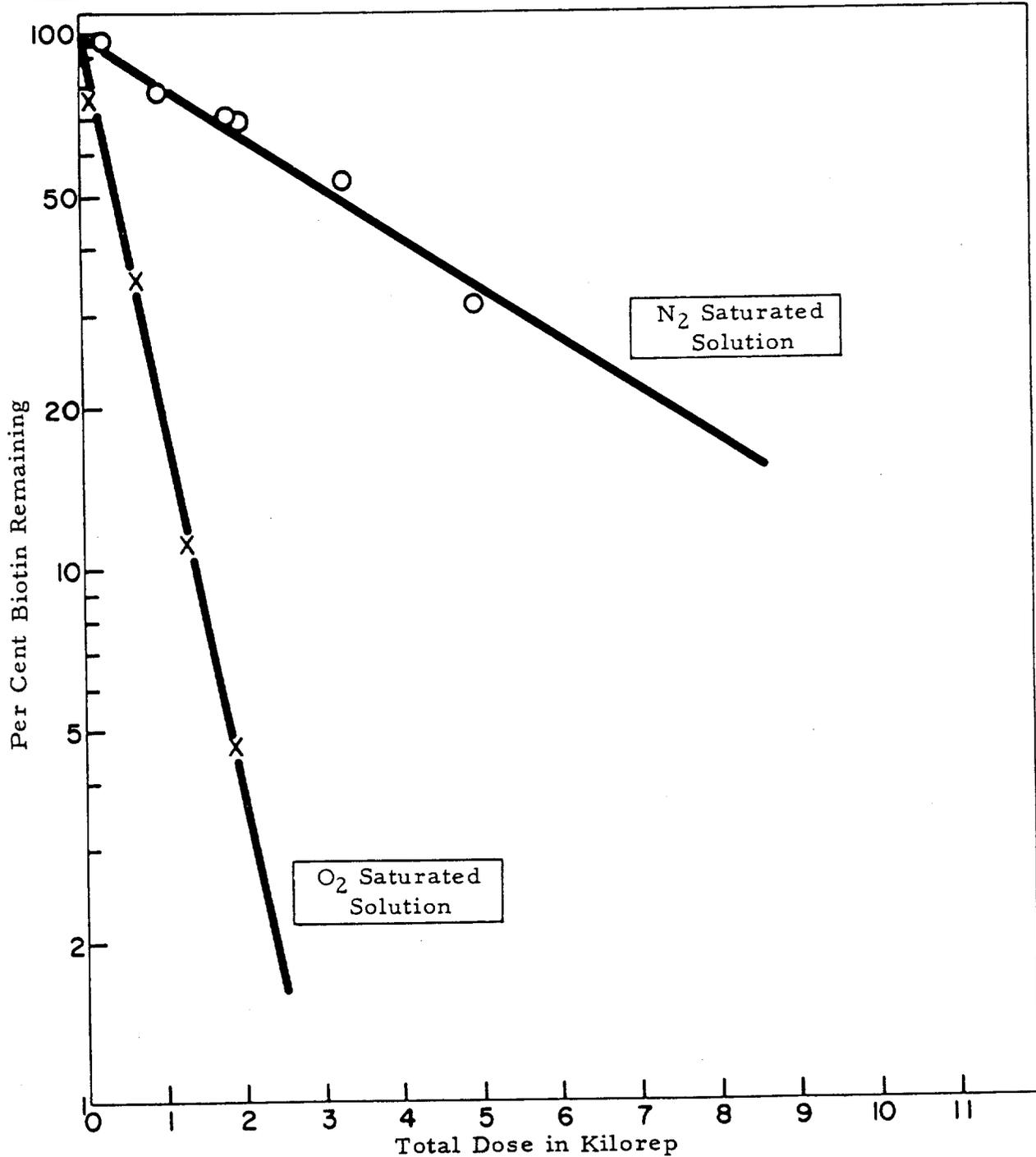


FIGURE 3
DESTRUCTION OF BIOTIN IN AQUEOUS SOLUTION BY TRITIUM
 β IRRADIATION. INITIAL BIOTIN CONCENTRATION 0.010 $\mu\text{g/ml}$.

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In assaying tritium-containing solutions, it is of importance to keep the concentration of the radioactive isotope below the level at which it affects the organism. In these experiments it was determined that at tritium concentrations below 5 mc/ml there was no alteration of growth, as measured by optical density, of the organism over the time interval employed for the assay.

TABLE 1

Values of Reaction Constants for Destruction of
Vitamins in Aqueous Solutions

	$-K(\text{Kilorep}^{-1})$	
	O ₂ Saturated	N ₂ Saturated
Riboflavin	.83	.32
Biotin	1.66	.22
Folic acid	1.91	.77

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Progress Report

AN AUTOMATIC RECORDING TURBIDIMETER

M. E. Getzendaner and R. R. Cone*

An instrument is described which automatically records turbidities of bacterial cultures during their growth.

The previously reported determinations of relative biological effectiveness using microbiological response to ionizing radiations (1) were carried out by manual determination of turbidity of the cultures. This procedure suffered from the disadvantages of many handlings, using special precautions to prevent radiation exposure, danger of spills, changes of temperature during the growth period, and the necessity for periodic readings requiring an operator on all night duty.

We now have obtained an automatic recording turbidimeter (Autoturbidimeter) which was designed to overcome these difficulties. The automatically recording turbidimeter described by Rubin (2) served as a starting point for the developments described here. A photograph of the instrument is shown in Figure 1. The rotating turntable, holding a maximum of 45 samples, brings each tube in turn into reading position in the light beam, and holds it stationary for several seconds by means of a Geneva wheel system. This sample-holding wheel makes a complete revolution in 10.5 minutes. Sample tubes are suspended in a doughnut-shaped tank through which water is pumped from a thermostated bath. A magnetic stirrer, located three positions before the reading position, activates a small Alnico magnet sealed in glass and contained in each tube. In this way the culture is uniformly suspended in the medium just before moving into reading position.

*Member of the Instrument Development Group, Design Section,
Engineering Department.

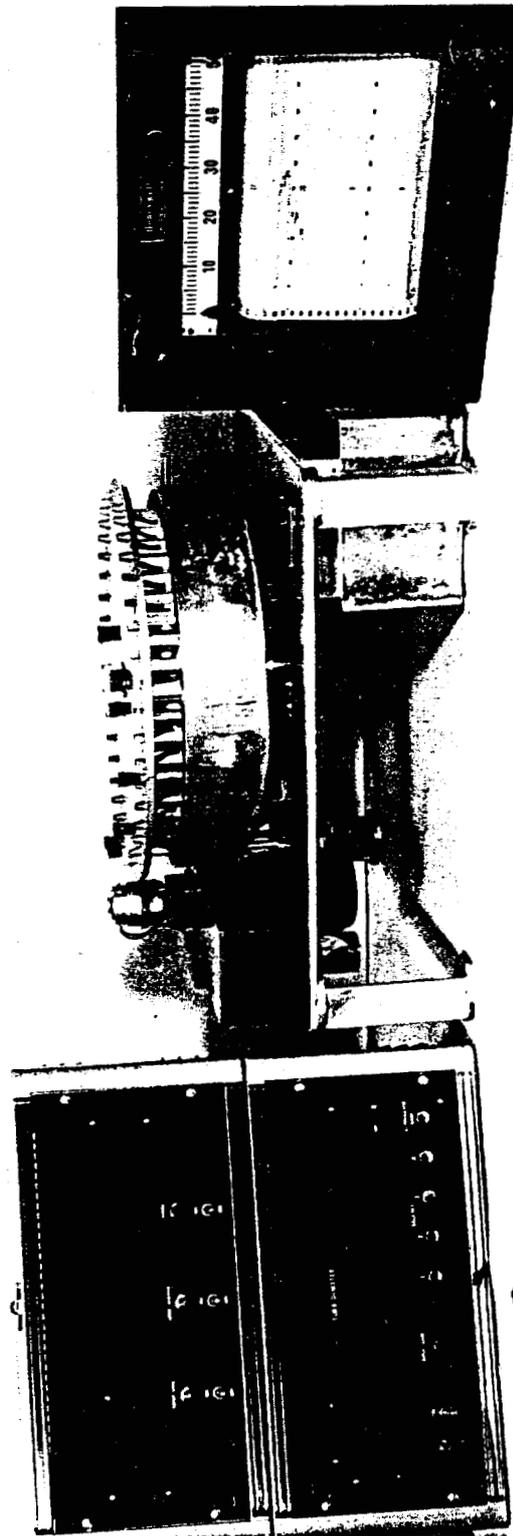


FIGURE 1. PHOTOGRAPH OF COMPLETE AUTOTURBIDIMETER.

The measurement of turbidity is accomplished by shining a light alternately through the sample and through a calibrated glass slide of varying optical density (optical wedge) to the phototube. The alternation is accomplished by means of the light chopper shown in Figure 2, which alternately cuts each beam coming from the light source through a beam splitter. The phototube produces pulses which are proportionate in height to the light intensity passing through the sample or glass slide. This difference in pulse height between sample and glass slide is amplified and detected in a discriminator unit. The output of the discriminator is fed to a power amplifier which drives a motor causing the glass slide to move to a point where the pulse from the light passing through the slide equals the pulse from the light passing through the sample. A null balance exists at this point and the turbidity can be determined by noting the position of the glass slide. This is accomplished by the glass slide drive cable acting on a helipot. A Brown recorder is activated by the helipot so that a tracing is made on a chart indicating the position of the glass slide, and hence the turbidity of the experimental tube at all times. Figure 3 shows a block diagram of the optical and electrical components, and their relationship to each other. A timing device turns the Brown recorder chart drive off for selected periods between recordings of complete cycles of the sample wheel. This permits intermittent recording of data and allows one chart to record all the readings of a single experiment lasting over 24 hours.

It is expected that this instrument will greatly facilitate the determination of the relative biological effectiveness of radiation by the microbiological method.

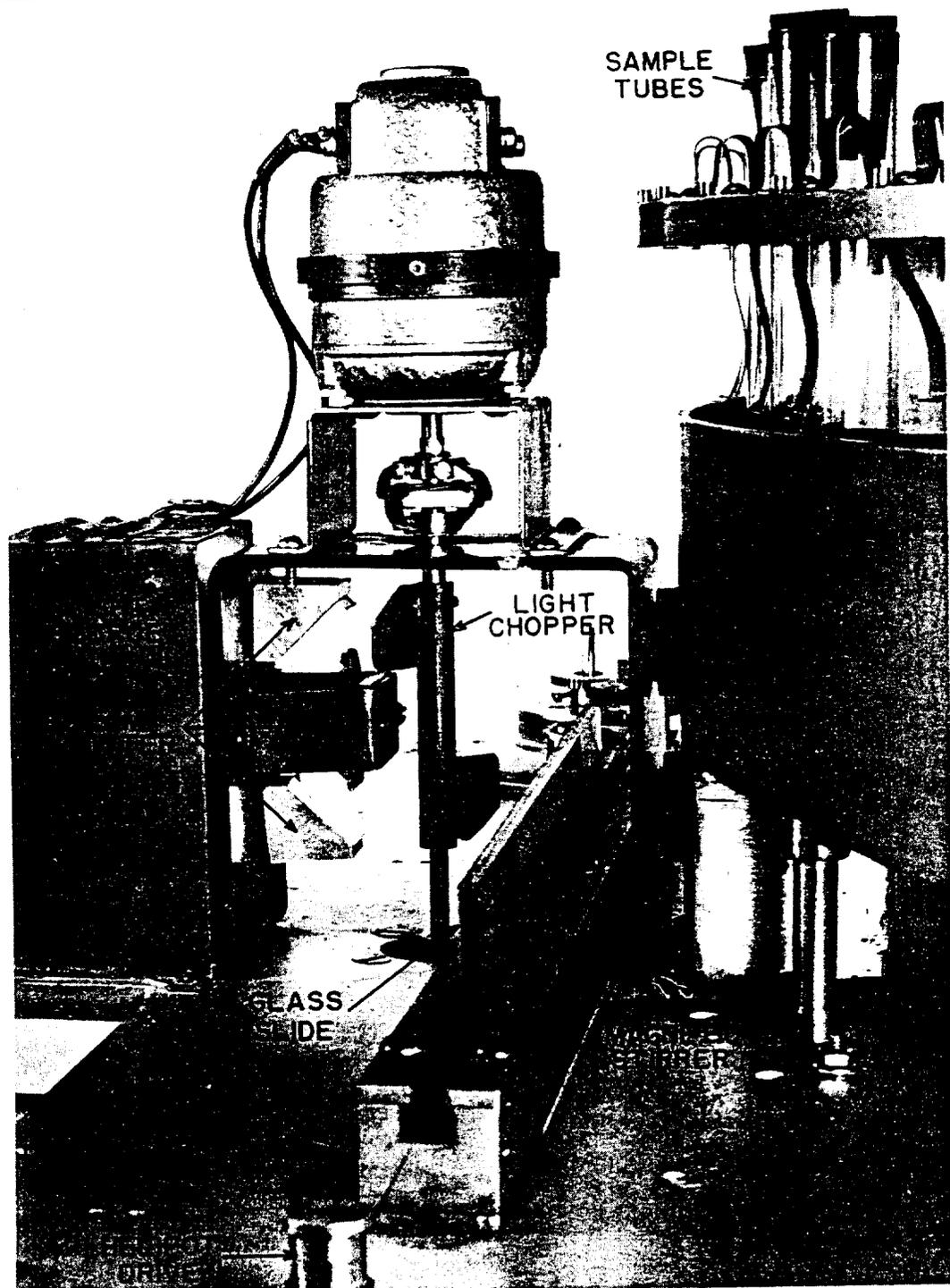


FIGURE 2. CLOSE-UP VIEW OF PART OF THE OPTICAL COMPONENTS OF THE AUTOTURBIDIMETER.

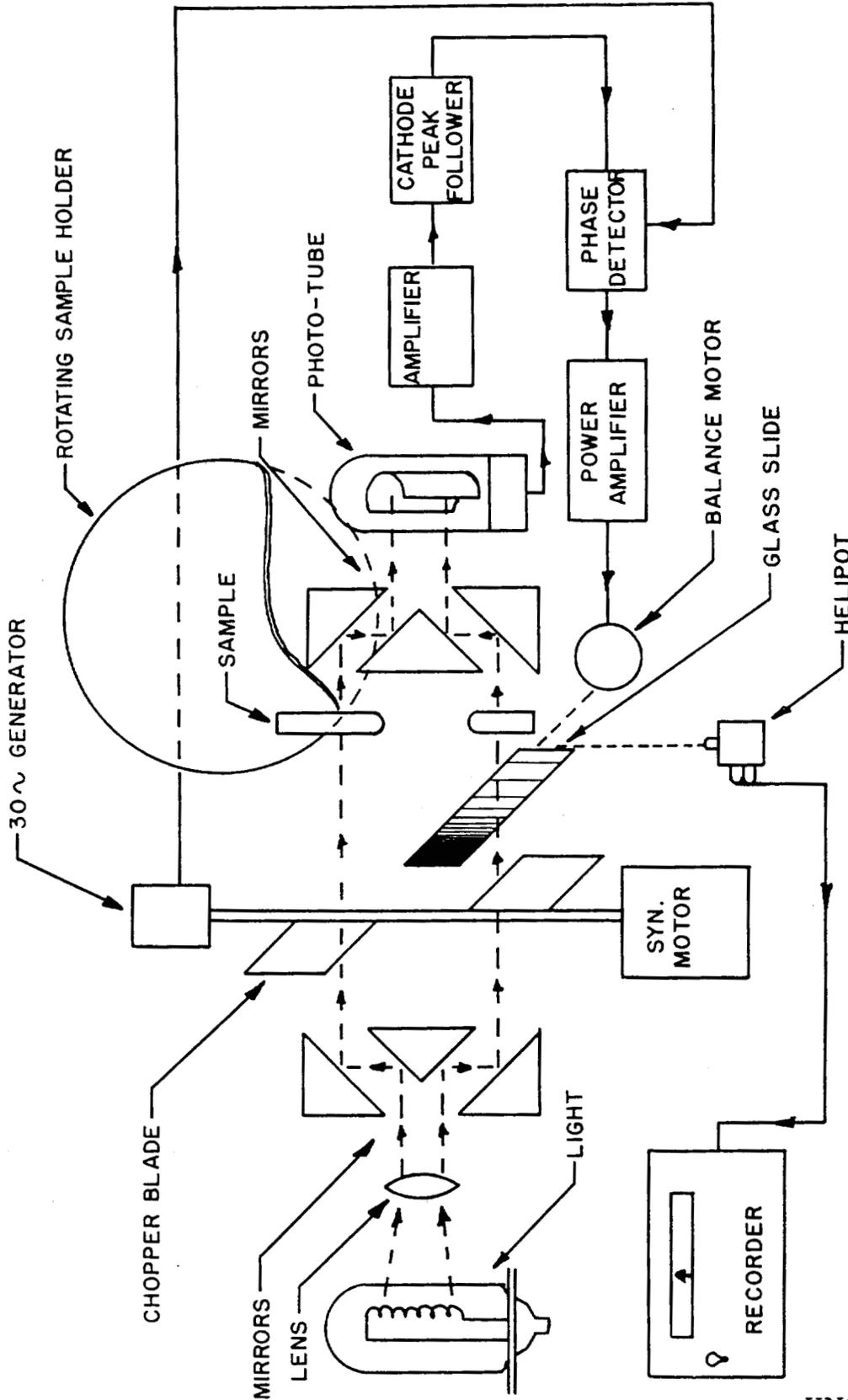


FIGURE 3
BLOCK DIAGRAM OF OPTICAL AND ELECTRICAL COMPONENTS OF AUTOTURBIDIMETER.

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Progress Report

MUTAGENIC EFFECTS OF P³²

F. P. Hungate, H. J. Dishburger and A. A. Selders

Experiments to evaluate the amount of biochemical mutation induced in the mold Neurospora crassa by the transmutation of P³² have yielded results which are not entirely conclusive. It appears, however, that there is some induction of mutation by transmutation as differentiated from that induced by radiation.

Several investigators have attempted to identify effects ascribable to transmutation, as well as those resulting from radiation, when metabolizable radioisotopes are used as a radiation source. Evidence has been presented that mutations were obtained by the transmutation of P³² in Paramecium (1) and bacteria (2), but no increase in lethal mutation rate as a result of transmutation was found in Drosophila (3). Extensive effects on killing of phage (4) have been related to the transmutation of P³². Transmutation of S³⁵ was found to induce biochemical mutation in Neurospora (5), but no such mutation effects were found in Paramecium when H³ (6) was the metabolized isotope.

The present investigation was undertaken to evaluate the effectiveness of P³² in inducing biochemical mutations in N. crassa. An isotope dilution technique was used to differentiate between the rate of mutation induced by transmutation as opposed to that induced by radiation.

MATERIALS AND METHODS

Stock cultures of Neurospora crassa, Strain Y8743, were grown on complete medium (8) and prior to use transferred to minimal medium containing only one per cent as much phosphorus as normally present. All cultures were grown at 27°C. Components of the test medium were as in standard minimal medium and varied only in phosphorus content as summarized in Table 1.

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TABLE 1

Phosphorus Content, Variation in Radiation at the Culture Level, and Biochemical Mutation Frequency

Number	P ³² /0.5 ml culture (μc)	Approximate Ratio P ³² /P ³¹	C ₄ /C ₀ *	No. Tested	Per Cent Mutation
8	20	1.6 x 10 ⁻⁶	1.4	297	0
9	20	8 x 10 ⁻⁶	1.9	298	0
10	20	5 x 10 ⁻⁵	2.2	374	1.1
11	20	1.7 x 10 ⁻⁴	0.7	300	1.7
12	20	5 x 10 ⁻⁴	0.8	29	3.0
13	20	1 x 10 ⁻³	0.9	0	
21	100	8 x 10 ⁻⁶	1.3	140	1.4
22	100	3.8 x 10 ⁻⁵	2.3	238	1.7
23	100	1.7 x 10 ⁻⁴	2.4	353	2.5
24	100	5 x 10 ⁻⁴	1.1	55	3.6
25	100	1 x 10 ⁻³	0.9	0	

*C₄ is the counting rate on the fourth day as measured by a GM tube.

C₀ is the counting rate at the time of inoculation.

Data reported here are from cultures grown in flat-bottom shell vials 16 mm in diameter. The 0.5 ml of medium formed a very shallow layer, allowing thus a high uniformity of radiation during growth. Following addition of the medium and P^{32} to the vials, they were capped and autoclaved at 15 lbs pressure for 10 minutes. Upon cooling, they were each inoculated with one drop of a light suspension of conidia from a culture grown on phosphorus deficient media.

A measure of the radioactivity at the surface of the medium was obtained by placing these cultures under a GM tube with a suitable absorber interposed to bring the counting rate into a countable range. Counts were made on successive days using standardized procedures so that the geometry and absorption factors remained constant. Comparisons of initial counts and those obtained on the fourth day are included in Table 1.

Following a growth and exposure period of 34 days, one ml of sterile distilled water was introduced into each vial, the vials agitated to suspend the conidia, the suspension filtered through a loose cotton plug, and the filtrate added to one ml of sterile water. Aliquots of this filtrate were plated on sorbose-complete plates (7). These plates were incubated until colonies appeared, at which time they were placed in a refrigerator to prevent further growth during the delay in isolating individual colonies. All discrete colonies in any plate were picked and placed in individual tubes. The individual strains were then tested first on minimal medium and then on three growth supplements according to the methods of Beadle and Tatum (8).

RESULTS AND DISCUSSION

Table 1 gives the per cent biochemical mutants recovered from the several cultures. It is apparent that the mutation rate increases in each series as the specific activity of the P^{32} increases. If the radiation delivered to the mold were the same in all cultures, these results would have to be interpreted as stemming from the effects of transmutation. If, however, the radiation delivered to the mold varied, the results might well derive from such variations in the amount of radiation.

Since the total amount of P^{32} added to each tube of a series was the same, it was expected that the radiation delivered to the mold cultures within a series would be the same except for effects of geometry and/or concentration resulting from uptake of phosphorus by the mold. If a considerable fraction of the phosphorus were taken up by the mold which grows on the surface, one would expect an increased count above the culture. Such an increase was observed by counting the tubes on successive days with a GM tube. The results of these counts are shown as the ratio of counts after four days to the initial counts. It is noteworthy that in each series this ratio tends to increase as the amount of phosphorus is decreased and then falls with further decrease of phosphorus. This increase is probably accounted for by uptake of a larger proportion of the phosphorus as it becomes more limiting. Further reduction of phosphorus, however, appears to retard phosphorus concentration in the surface area, probably by severely restricting growth.

There is a slight increase in mutation rate in those cultures receiving 100 μ c of P^{32} over those receiving 20 μ c, but the increase is neither the fivefold increase expected as a consequence of the increase in radiation nor the fivefold increase expected from the higher specific activity.

The interpretation of the data reported here, as is true also with that from similar experiments using modified culture containers, is clouded by the simultaneous variation of both the specific activity of the P^{32} and radiation delivered to the mold. It appears, however, that the mutation rate continues to rise in relation to the increased specific activity even where the radiation falls off, as in cultures number 11, 12, and 24. This indicates that transmutation is causing biochemical mutations to occur. It is surprising that the effect observed is so slight, since the large phosphorus content in the nucleic acid portion of the chromosome leads one to expect changes even at relatively low specific activities.

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Abstract

GROSS EFFECTS OF GROWTH-INHIBITING LEVELS OF
TRITIUM OXIDE ON CHLORELLA PYRENOIDOSA

J. W. Porter and M. S. Watson

The studies here reported are an extension of work described in the previous annual report (1). A detailed account of the methods employed and the results obtained in these studies appear in HW-30056 (2).

The mass of cells formed at the end of three days' growth in the presence of tritium oxide, and at the end of three days' subsequent growth in subculture in non-radioactive nutrient solution, was an inverse function of the amount of irradiation received by the cells. When irradiated cells were subcultured repeatedly in non-radioactive nutrient solution, it was found that the culture returned to the same growth rate as the control. Cells grown three days in the presence of 5 mc/ml of tritium oxide required one subculture of three days to return to normal growth rate, whereas those grown three days in the presence of 15 mc/ml of tritium oxide required two subculture periods.

The number of Chlorella cells formed in the presence of tritium oxide and in subsequent subculture in organic and inorganic nutrient solution was also an inverse function of the dose of radiation received by the cells. Cell size increased as a consequence of irradiation.

Viability of cells after a three-day growth period in the presence of tritium oxide was an inverse function of the dose of radiation received by the cells. When cells were grown three days in the presence of 20 mc of tritium oxide/ml, approximately 73 per cent of the cells were unable to form colonies when plated. Growth assays of other aliquots of the cells showed that death of the cells occurred gradually after they were plated. Viability of the irradiated Chlorella could not be increased by adding glucose,

yeast extract and bacto peptone to the nutrient solution. From this result it was concluded that ionizing radiations do not selectively damage the processes of photosynthesis or the synthesis of the substances present in bacto peptone and yeast extract.

Assays of selections from a sector of colonies of irradiated Chlorella for growth rates showed that 25 per cent of the cells surviving irradiation were changed genetically. These cells grew significantly more slowly than control cells. Among the selections surviving irradiation were a biochemical mutant, several rough selections, lightly pigmented selections and selections which clumped and sedimented badly when grown in liquid culture.

The results suggest that in Chlorella the process of cell division is damaged to a greater extent than the processes involved in growth. The major effect of ionizing radiations on Chlorella is death of the cell, which occurs gradually, and is not caused by selective action of the ionizing radiations on the processes of photosynthesis or the biosynthesis of the substances present in bacto peptone and yeast extract.

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Progress Report

THE EFFECT OF TRITIUM OXIDE ON SOME
SYNTHETIC PROCESSES OF CHLORELLA PYRENOIDOSA

J. W. Porter and H. J. Knauss

Chlorella previously grown in inorganic media containing tritium oxide were subcultured in media containing C^{14} -labeled bicarbonate. Incorporation of C^{14} into fatty acids was markedly increased in the previously irradiated cells. Nucleic acid synthesis was also increased, and chlorophyll a synthesis decreased. Further experiments with C^{14} -labeled acetate indicate certain similarities in the metabolic behavior of previously irradiated algae and algae growing in organic media.

In an effort to gain some insight into the mechanism of radiation damage in algae, studies were made of the changes in synthetic activity in algae following irradiation. Previously irradiated algae were subcultured in the presence of C^{14} -labeled bicarbonate and the incorporation of C^{14} into various compound fractions was compared with the incorporation of C^{14} by control cells. A complete description of the experimental procedure and a more detailed account of some of the data here presented will be found in HW-30252 (1).

The quantities of C^{14} incorporated into cellular components of previously irradiated and previously unirradiated Chlorella during a 30-hour growth period in the presence of C^{14} -labeled bicarbonate are shown in Table 1. Values shown are those obtained from single cultures, however, similar results were obtained from duplicate cultures in this and three similar experiments. The most striking effect of radiation is the increased C^{14} incorporation in fatty acids. Although not obviously significant from the data in Table 1, C^{14} incorporation into the nucleic acid fraction was consistently increased as a consequence of radiation, and C^{14} incorporation into the chlorophyll a fraction was consistently decreased.

TABLE I
 Incorporation of C¹⁴ into Various Chlorella
 Fractions during a 30-Hour Growth Period

	C ¹⁴ -Labeled Bicarbonate		C ¹⁴ -Labeled Acetate	
	Not Irradiated Inorg. Med.	Irradiated Inorg. Med.	Not Irradiated Inorg. Med.	Not Irradiated Org. Med.
Original Optical Density	.21	.21	.26	.26
Final Optical Density	1.10	.84	.94	1.4
Cell mass doublings	2.3	2.0	1.8	2.3
<u>Cell Fraction</u>	d/m x 10 ⁻⁶			
Whole cells	16	17	22	26
Methanol extract	--	4.6	8.1	16
Methanol insolubles	11	10	10	7.2
Ether extract	2.1	2.5	5.7	12.3
Methanol sol., ether insol.	1.6	1.7	2.4	2.8
β-carotene and triglycerides	.13	.29	.12	2.3
Luteol	.16	.16	.11	.17
Chlorophyll a	.34	.29	1.0	.54
Chlorophyll b	.09	.09	.34	.18
"Above Chlorophyll b"	.38	.39	1.8	3.1
Non-saponifiables (β-carotene)	.064	.066	.017	.027
Fatty acids	.039	.17	.069	1.2
Butanol solubles	.010	.013	--	--

TABLE 1 (contd.)
 Incorporation of C¹⁴ into Various Chlorella
 Fractions during a 30-Hour Growth Period

	C ¹⁴ -Labeled Bicarbonate		C ¹⁴ -Labeled Acetate	
	Not Irradiated Inorg. Med.	Irradiated Inorg. Med.	Not Irradiated Inorg. Med.	Not Irradiated Org. Med.
			d/m x 10 ⁻⁶	
Unsaturated fatty acids	.007	.019	--	--
Saturated fatty acids	.0009	.006	--	--
NaCl extract (nucleic acids)	.28	.34	.33	.38
NaCl insolubles	9.6	10	--	--
Trypsin digest (protein)	1.9	2.0	5.9	3.1
Resistant to trypsin	4.6	4.3	2.7	2.5
1% HCl soluble (starch)	2.0	1.9	--	.14
Acid insoluble (cellulose)	2.0	1.6	--	.86

Rather surprisingly, a number of processes (protein, starch, and carotenoid synthesis) are not affected, at least at the gross level of analysis employed, by concentrations of tritium oxide that inhibit growth and cell division.

Also shown in Table 1 is a comparison between C^{14} incorporation from labeled acetate by algae, not previously irradiated, growing in inorganic and organic media. The main point of interest in these data is the similarity between the incorporation of C^{14} by algae growing on organic media, and by irradiated algae growing on inorganic media. Thus, algae grown on organic media as compared with algae grown on inorganic media, incorporated much larger amounts of C^{14} into fatty acids, larger amounts into the nucleic acid fraction, and smaller amounts into the chlorophyll a fraction. Additional differences are noted in the labeled acetate experiments which were not observed in the irradiated algae, so the analogy is not complete.

These studies have not progressed to the point where any conclusions can be drawn concerning the mechanism of the effects noted. It is tempting to speculate, however, on certain possible explanations. Selective radiation damage to terminal oxidases would explain a shunting of hydrogen, normally oxidized to water, to two carbon acceptors which by conversion to acetate could lead to increased fat synthesis. Many other explanations are possible and the apparent similarity between radiation damaged algae and algae growing on organic media may aid in choosing between these possibilities.

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Abstract

INCORPORATION OF HYDROGEN ISOTOPES INTO
VARIOUS FRACTIONS OF RAPIDLY GROWING ALGAE

J. W. Porter

Experiments demonstrating an isotope effect in the incorporation of tritium, deuterium, and protium into Chlorella pyrenoidosa cells were described in the previous annual report (1) and in the open literature (2). Further studies have shown that this effect occurs to a varying extent in different fractions separated from the algal cells. Details of these studies appear in HW-29193 (3).

Following growth in media containing deuterium and tritium oxides, algal cells were separated into methanol insoluble, methanol soluble - ether soluble, and methanol soluble - ether insoluble fractions. Results of tritium, deuterium, and total hydrogen analyses on these fractions are interpreted in terms of isotope effect in Table 1. The extent of tritium incorporation is slightly, but significantly lower than deuterium incorporation, except in the case of the methanol soluble - ether insoluble fraction, where the difference is not statistically significant. Both tritium and deuterium are incorporated to a markedly lesser degree than protium, the magnitude of the effect varying significantly between the various fractions.

TABLE 1

Comparison of Tritium, Deuterium and Protium Incorporation
into Separated Fractions from Algal Cells

Cell Fraction	No. of Analyses	Relative Extent of Incorporation Compared to Protium (%)		Ratio of Extent of Incorporation T/D*
		Tritium*	Deuterium*	
Whole cells	10	45 ± 2	50 ± 2	0.89 ± 0.05
Methanol insoluble	10	42 ± 2	45 ± 4	0.93 ± 0.08
Ether soluble	10	55 ± 5	63 ± 3	0.88 ± 0.08
Ether insoluble	5	38 ± 8	32 ± 5	1.17 ± 0.23

*Averages ± 95 per cent confidence limits.

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Abstract

COMPARISON OF THE METABOLISM OF DEUTERIUM
AND TRITIUM IN THE RAT

R. C. Thompson and J. E. Ballou

In the previous annual report preliminary partial results were given for an experiment which involved a comparison of deuterium and tritium incorporation and retention in rats (1). This experiment was completed and detailed findings reported in HW-28595 (2).

Thirty-two rats were administered tritium oxide and deuterium oxide in a constant ratio over a five-week period. Animals were sacrificed in eight groups over a two-month period and the ratio of bound tritium to bound deuterium determined in various tissues. For body water, total carcass, pelt and liver, there was no significant difference between the ratio of bound tritium to bound deuterium and the ratio of tritium to deuterium in the solution injected. Significant differences of six to seven per cent, favoring deuterium incorporation, were observed in the case of muscle, fat and brain. There was no consistent trend of change in ratios for a given tissue over the 60-day period. It was therefore concluded that the observed isotope effects were exhibited in the initial incorporation of the isotopes.

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Progress Report

DISTRIBUTION OF BOUND TRITIUM IN THE RAT
FOLLOWING EXPOSURE TO TRITIUM GAS

G. N. Smith*, O. L. Hollis and R. C. Thompson

Procedures were devised for exposing rats to relatively high concentrations of tritium gas, under conditions which minimize the possibility of tritium oxide formation in the environment. Preliminary data on distribution of tritium within the animal following such exposures are presented.

Present knowledge of the distribution and retention of tritium in the animal organism has been derived almost exclusively from experiments in which the tritium was administered in the form of the oxide. There is some indication that tritium administered as the elemental gas may be distributed in a different manner, and that a higher proportion of the tritium retained following exposure may be in the organically-bound state (1).

In the present study rats were exposed under conditions which result in the incorporation of a sufficient concentration of tritium (as the oxide, or organically-bound) to permit a study of its retention over a long period following exposure. Conditions of exposure were also designed to insure the absence of tritium oxide in the exposing atmosphere, since the conversion of a small fraction of the tritium gas to tritium oxide, within the exposure system, would greatly influence results obtained in the animals.

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METHODS

The exposure system which was eventually adopted is shown in Figure 1. It is a closed system through which the atmosphere is circulated by means of a "Sigmamotor" finger pump which operates by squeezing the atmosphere through a length of polyethylene tubing. The atmosphere thus comes in contact with no metal parts of the pump. The atmosphere first passes through a NaOH bubbler (B) which removes CO₂ expired by the rats. Oxygen removed by the rats is replaced at (C) where a continuously flowing stream of oxygen is admitted to the system when the pressure within the system drops. The atmosphere next passes through a large drying tube (D) filled with magnesium perchlorate and calcium sulfate. It then passes through an automatic recording ionization chamber, to monitor tritium content which is flanked on each side by U-tubes (E) immersed in a dry ice-acetone bath. The section (G) is used for introduction of tritium, and may be replaced by sampling bulb (H). The exposure chamber (J) is a modified vacuum desiccator in which the animals are supported above a layer of magnesium perchlorate and calcium sulfate. The exposure chamber will accommodate from four to six large rats. Circulation time in the system is about five minutes.

RESULTS

Thus far results have been obtained on four rats exposed for four hours to an atmosphere containing 200 μ c tritium per cc. These animals were sacrificed an hour after the end of the exposure and tritium determined in the tissue water and dry organic material of a variety of samples. Results of these determinations are shown in Table 1.

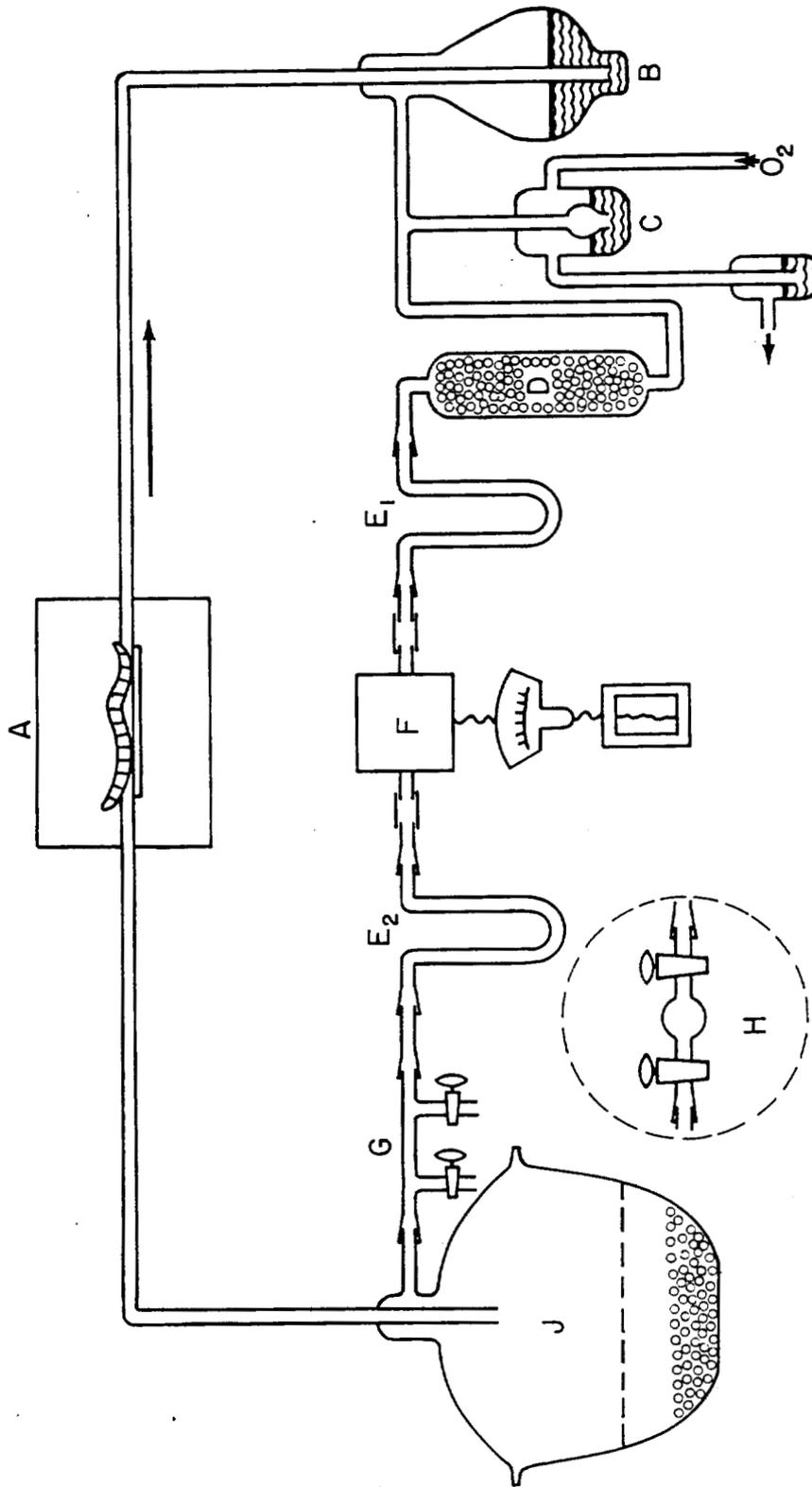


FIGURE 1
APPARATUS FOR EXPOSURE OF RATS TO TRITIUM GAS.

TABLE 1
Incorporation of Tritium Following Exposure of Rats to Tritium Gas or Tritium Oxide

Tissue	Gas-Exposed Animals*		Oxide-Exposed Animals**
	Tritium Oxide ($\mu\text{c}/\text{ml}$ tissue water)	Bound Tritium (% of avg. HTO conc.)***	
Liver	69	4.4	2.7
Spleen	49	4.2	
Kidney	62	2.7	1.0
Heart	43	2.5	0.6
Brain	63	2.2	0.5
Lung	58	1.8	0.7
Muscle	60	1.2	0.13
Lg. Intestine	59	1.2	1.0
Stomach	70	1.1	1.2
Sm. Intestine	62	0.9	1.9

* Animals sacrificed one hour after a four-hour exposure to tritium gas.

** Animals sacrificed one day after a single intraperitoneal injection of tritium oxide (2, 3).

*** $\text{Bound tritium conc. } (\mu\text{c/g moist tissue}) \times 100$
Average initial tritium content of body water ($\mu\text{c}/\text{ml}$)

Average initial tritium content of body water was 60 $\mu\text{c}/\text{ml}$ for gas-exposed animals and 127 $\mu\text{c}/\text{ml}$ for oxide-exposed animals.

DISCUSSION

The data thus far obtained are of a very preliminary nature and serve only to indicate the possible trends to be anticipated in further studies. Some difficulties were experienced with the routine tritium analyses during the period of this study, which adds an additional uncertainty to the results obtained. Further studies will involve the exposure of larger numbers of animals which will be sacrificed at intervals following exposure.

The distribution of tritium oxide among the various tissues sampled, following exposure to tritium gas, is reasonably uniform, considering the conditions under which the analyses were obtained. The relatively low values obtained for heart and spleen cannot be considered significant without further verification.

The concentration of organically-bound tritium in the animals exposed to tritium gas is expressed in Table 1 as a percentage of the average concentration of tritium oxide in body water. This is done to facilitate comparison with earlier studies (2, 3) in which organically-bound tritium was determined following intraperitoneal injection of tritium oxide. Values from these earlier studies, similarly expressed, are shown in the last column of Table 1. The comparison between these two types of exposure is not a completely valid one, since the gas-exposed animals were sacrificed an hour following exposure, while the oxide-exposed animals were sacrificed a day following exposure. Had the oxide-exposed animals been sacrificed earlier, higher levels of organically-bound tritium might have been observed in certain of the tissue, however, the level would undoubtedly have been lower in some of the tissues, such as lung and muscle, which showed a higher organically-bound tritium level six days after tritium oxide injection than they did one day following injection (2, 3).

Despite the reservations noted above, it seems evident that in certain tissues the metabolic incorporation of tritium, as compared to tritium oxide retention, is greater following exposure to tritium gas than it is following administration of tritium oxide. This effect is especially marked in the muscle sample. This may be an indication that appreciable amounts of tritium are directly incorporated into tissue compounds without passing through the stage of tritium oxide.

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Progress Report

DISTRIBUTION OF BOUND TRITIUM IN THE RAT
FOLLOWING CHRONIC EXPOSURE TO TRITIUM OXIDE

R. C. Thompson and J. E. Ballou

Mature female rats were maintained on a constant, tritium oxide-labeled drinking water diet for four months. Six weeks after being placed on this regimen they were mated and the young weaned to the same tritium-labeled drinking water. The original females are being sacrificed at intervals following removal from the tritium oxide regimen. The second generation animals were removed from the tritium oxide regimen at the age of six months and are being sacrificed at intervals. Average tritium concentration in tissue-bound hydrogen in both groups of animals at the end of the exposure period was approximately 20 per cent of the tritium concentration in body water hydrogen.

In our previous study of long-term retention of tissue-bound tritium in the rat, tritium oxide was administered as a single, intraperitoneal dose (1, 2). From this study it was deduced that certain widely distributed body components are metabolically quite inert, the tritium in these components being lost from the rat with biological half-lives ranging from 80 to 300 days. The size of these stagnant metabolic pools could only be inferred on the basis of many assumptions relative to the processes involved in the incorporation and release of tritium from the compounds involved. A direct measurement of the size of these pools would be possible if one had animals in which all hydrogen was uniformly labeled with tritium. In such animals the size (or more precisely, the hydrogen content) of any isolated component would be proportional to its tritium content.

The present experiment is an attempt to approach this ideal experimental animal by raising rats from conception in a body water environment of constant tritium oxide concentration. In addition to the information

which may be obtained from these animals regarding the size and turnover rates of metabolic pools, they permit a direct measurement of the increased radiation occasioned by bound tritium in animals chronically exposed to tritium oxide.

METHODS

Twelve mature, Sprague-Dawley, female rats, averaging 235 g, were injected intraperitoneally with an amount of tritium oxide solution calculated to bring their body water tritium concentration to 3.5 $\mu\text{c}/\text{ml}$. All drinking water subsequently supplied to these animals contained 5.0 μc tritium oxide per ml. Purina dog chow was fed ad libitum throughout the experiment. Approximately six weeks after the institution of this regimen the animals were bred. Ten of the females delivered young, 38 of which survived the weaning. Mortality at birth was high due to cannibalism. Control females bred at the same time failed to conceive. Eighteen young rats, born about a month later than the experimental animals, were used as weight controls. The experimental young were weaned to the same tritium oxide drinking water regimen as was employed for the mothers.

The mothers were removed from the tritium oxide regimen four months after its institution. One group of two animals was sacrificed immediately and other groups sacrificed at 10, 30, and 93 days. Four of these original animals remain to be sacrificed at later periods. The young rats were removed from the experimental regimen at the age of six months. Groups of three male and three female rats were sacrificed immediately, and other groups sacrificed at 5, 14, and 35 days. Additional groups will be sacrificed at later periods. Samples of various organs and tissues were removed from the animals at sacrifice, pooled for a given group and analyzed for tissue-bound tritium. Various compound fractions will also be isolated and analyzed.

RESULTS AND DISCUSSION

Analyses on urine samples collected during the period of tritium oxide feeding, and body water tritium content determined at sacrifice of the initial groups of both first and second generation animals, indicated a fairly constant body water tritium content of about 3.6 - 3.9 $\mu\text{c}/\text{ml}$. This would result in an average total body radiation dose from body water of about 0.8 rad/day. Weight curves of the experimental second generation animals lagged somewhat behind the controls. At the age of 130 days both male and female experimental groups were about 25 g lighter than the controls at the same age. No other evidence of detrimental effect of radiation was obtained, and, as pointed out above, the controls were of doubtful significance since they were born a month later than the experimental animals.

Analyses have been completed on tissue and organ samples of only the earliest groups sacrificed. No data are yet available on the tritium content of separated compound fractions. Data on groups sacrificed immediately upon removal from the tritium oxide regimen are shown in Table 1.

On the average it would seem that the animals exposed from conception to the tritium oxide regimen accumulated about the same concentration of bound tritium as did the mature animals exposed for a four-month period. There were notable exceptions, however, e. g., brain and heart. The full interpretation of these data must await results from animals sacrificed at intervals following cessation of tritium oxide exposure. These results will permit resolution of the bound tritium into components of different biological half-life, and more striking differences between the first and second generation animals may be observed in comparisons of

TABLE 1

Tritium Concentration in Organically-Bound Hydrogen from Rats
Chronically Exposed to Tritium Oxide
(Expressed as per cent of tritium concentration in hydrogen of body water)

Sample	1st Generation	2nd Generation	
	Females	Females	Males
Residual carcass	22	18	19
Liver	28	26	27
Skin	25	24	28
Kidney	24	27	26
Brain	23	37	37
Muscle	23	19	25
Bone	19	17	16
Sm. Intestine	19	20	20
Stomach	17	25	24
Lung	17	25	24
Large Intestine	16	20	20
Fat	15	12	11
Heart	8	23	21

these components. Partial results from later groups of the first generation animals indicate, as would be expected, a much higher proportion of bound tritium of long biological half-life than was observed in the earlier, single injection experiment (1, 2).

Certain interesting conclusions may be drawn from the data presently available. Apparently an average of about 20 per cent of organically-bound hydrogen in the rat, is derived from body water. The remaining 80 per cent is presumably derived from preformed organic molecules ingested as food and incorporated without labilization of hydrogen.

From the relative proportions of tritium oxide and organically-bound tritium present in various tissues, one can determine the increase in radiation hazard occasioned by the metabolic incorporation of tritium. For the residual carcass sample from the first generation animals the pertinent data are: 0.20 μc bound tritium/g moist tissue, and 2.2 μc body water tritium/g moist tissue. As a consequence of tritium incorporation the radiation hazard is therefore increased by a factor of about nine per cent. Considering the long period of exposure, and the close agreement between first and second generation animals, the average bound tritium concentrations observed must closely approach equilibrium values. The per cent increase in hazard can therefore be considered a maximum value for chronic exposure. Individual tissues or compound fractions may, of course, greatly exceed this average value.

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Abstract

EFFECT OF BODY DISTRIBUTION AND RETENTION
OF TRITIUM ON THE HAZARD OF EXPOSURE TO TRITIUM OXIDE

R. C. Thompson

Recommendations concerning the maximum permissible amount of tritium in the human body have all been based on the assumption of its uniform distribution within the body, its presence as tritium oxide, and its loss from the body at the same rate as body water (1, 2, 3). Based on a number of animal studies it has been possible to arrive at certain conclusions regarding the effect of nonuniform distribution of body water and of long-term retention of tissue-bound tritium on the hazard of internal exposure to tritium oxide. A detailed account of the manner in which these conclusions were reached is contained in HW-30340 (4). The conclusions themselves are briefly summarized below:

1. Variation in water content of tissues will result in radiation dosage to certain tissues which is as much as 25 per cent higher than the dose calculated on the assumption of uniform tritium distribution in the animal.
2. Studies of bound tritium retention in organs and tissues of the rat indicate an average increase in radiation dosage, over that due to tritium oxide in body water, of 5 to 10 per cent. Highest bound tritium dosage was observed in fatty tissues.
3. Radiation from bound tritium in certain compound fractions from rats may be as high as 50 to 100 per cent of the radiation received from tritium in body water. The interpretation of these data is uncertain due to lack of precise information concerning the micro environment of these compounds in the intact animal.

4. Studies of bound tritium retention in a single sheep indicate a minimum increase in radiation exposure due to bound tritium of about 50 per cent in certain fatty tissues, and a minimum increase of 1 to 16 per cent in other tissues.

5. In rats exposed chronically to tritium oxide from conception to the age of six months, radiation dosage from bound tritium averaged nine per cent of the radiation received from body water tritium. The highest radiation dosage from bound tritium was delivered to abdominal fat and amounted to 19 per cent of the average total body radiation from tritium oxide in body water.

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Progress Report

ABSORPTION AND DISTRIBUTION OF RUTHENIUM IN THE FOWL

W. C. Hanson and R. L. Browning

Limited research in metabolism of ruthenium in fowl was undertaken due to possible differences between birds and mammals in metabolism of the isotope and the apparent lack of information on ruthenium metabolism in the fowl. Maximum concentration occurred at 24 hours, at which time approximately three per cent of the administered dose was present in assayed tissues, principally in kidney, liver and skeleton. Kidney and liver were apparently equally critical, exhibiting nearly equal concentration and rate of loss.

METHODS

Forty New Hampshire Red pullets were individually fed 250 to 280 microcuries of Ru¹⁰⁶, as ruthenium chloride, containing 0.026 mg ruthenium carrier, by direct pipetting into the crop. The birds were then confined in cardboard boxes fitted with false bottoms made of mesh wire to prevent recontamination by excreted ruthenium and mesh wire tops for ventilation. At varying time intervals, from one hour to twenty-one days, the animals were sacrificed and samples of the following organs were mascerated on stainless steel plates, dried at room temperature, and counted directly with a mica-window beta counter: kidney, liver, blood, muscle, skeleton, spleen, pancreas, ovary, brain, heart, lung, bile, thyroid, thymus, adrenal, skin and bone marrow.

RESULTS AND DISCUSSION

Maximum ruthenium concentration occurred in soft tissues 24 hours after administration of the isotope (Figure 1). The kidneys and liver contained the highest ruthenium concentration at this time. It would appear from these results that liver and kidney may be equally critical organs in the fowl. Approximately three per cent of the total administered ruthenium was found in the assayed tissues 24 hours after administration. This indicates a much higher rate of absorption from the gut than was previously reported for rats (1).

Table 1 shows the maximum ruthenium concentration attained in each of the tissues assayed. The skeleton reached its maximum ruthenium concentration 36 hours after administration, at which time it contained more than one per cent of the administered dose. Relatively low amounts were present in bone marrow. Muscle, of prime importance from a health hazard standpoint in waterfowl contamination problems, was relatively low in ruthenium, and yet, in total, contained one-third the amount of isotope in the skeleton due to the greater muscle mass (~47 per cent of total body weight). Brain exhibited the least affinity for the isotope of all organs sampled. Eggs laid during the experiment exhibited a 16:4:1 ruthenium concentration ratio in yolk, shell and albumen.

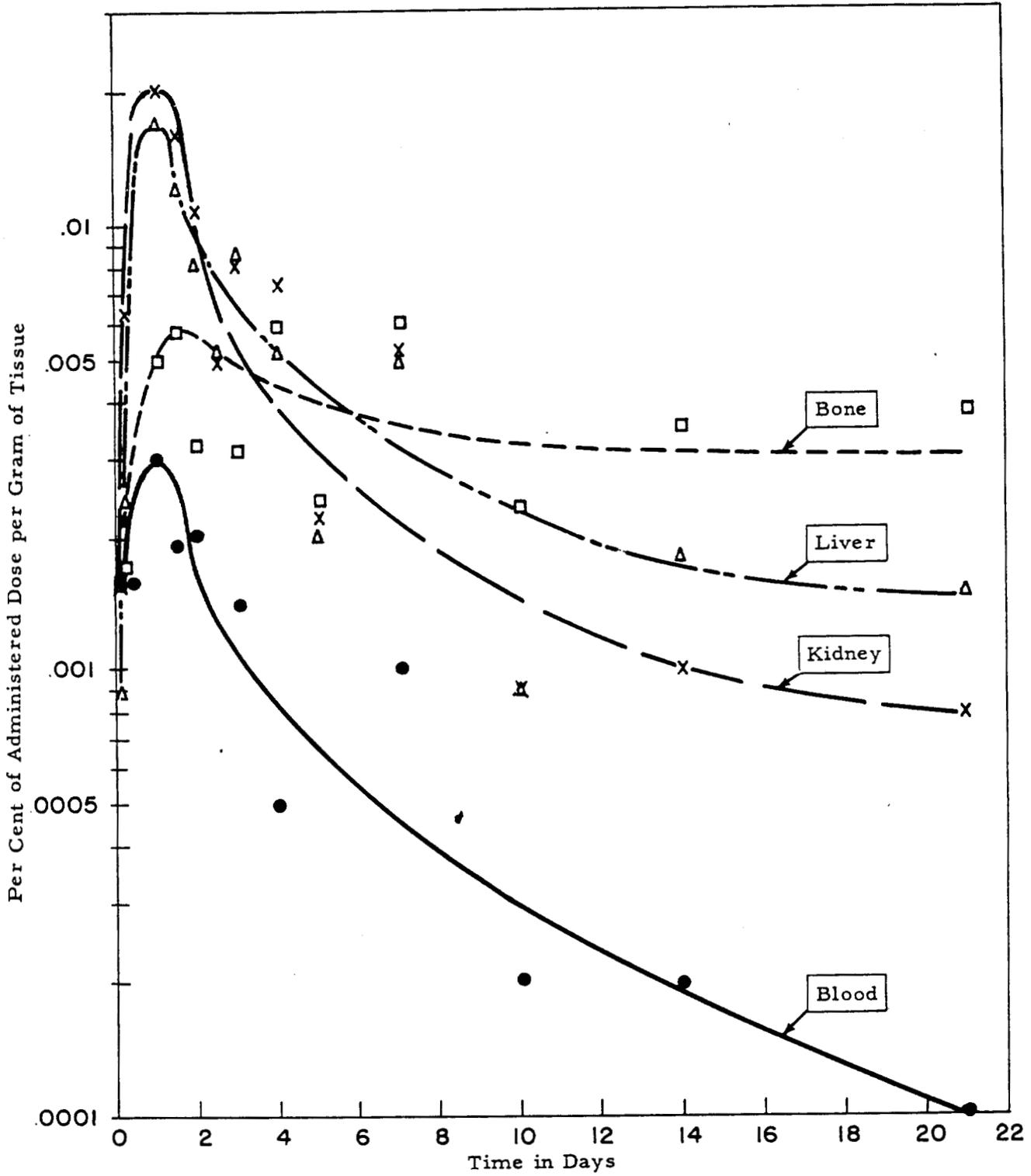


FIGURE 1
RUTHENIUM CONCENTRATION IN SELECTED TISSUES OF FOWL,

TABLE 1

Relative ruthenium concentration of various organs of the chicken at maximum uptake. Values in per cent of administered dose per gram of tissue.

Kidney	.020	Spleen	.0016
Liver	.017	Muscle	.0014
Skeleton	.0057	Heart	.0012
Lung	.0045	Thymus	.0012
Blood	.0030	Thyroid	.0010
Ovary	.0019	Marrow	.0010
Skin	.0019	Pancreas	.0009
Adrenals	.0017	Brain	.0001

Curves presented in Figure 1 are drawn to a minimum of points after three days and are therefore somewhat conjectural. Most soft tissues, other than kidney and liver, lost ruthenium with a biological half-life of 15 to 20 days. The rate of loss of ruthenium from the skeleton was notably slower, while loss from liver and kidney occurred at a half-life of approximately five days. One per cent of the administered dose was still present in the tissues after 21 days, the majority of this being in the skeleton.

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Progress Report

NORMAL ABUNDANCE OF RADIUM IN CADAVERS
FROM THE PACIFIC NORTHWEST

R. F. Palmer

Results of analyses on 50 cadavers collected in the Pacific Northwest indicate an average radium content of $(0.47 \pm 0.10) \times 10^{-10}$ g/body.

In order to establish a value for the mean body radium content for humans with no known occupational exposure, analyses of aliquots of cadaver ashes by a deemanation method previously described (1) have been continued. Analyses for radium have been completed on samples from a total of 50 cadavers collected in the Pacific Northwest.

The average total body radium content, with 90 per cent confidence limits was $(0.47 \pm 0.10) \times 10^{-10}$ g. Although this value is only 40 per cent of that reported by Hursh and Gates (2) for samples from the vicinity of Rochester, New York, in light of Hursh's values for the radium content of public water supplies (3), this result appears quite reasonable. The two Northwest cities, Portland, Oregon, and Tacoma, Washington, whose water supplies were analyzed by Hursh, gave values of 0.01×10^{-16} and 0.00×10^{-16} g radium per ml tap water, respectively. When these values are compared to 0.36×10^{-16} g radium per ml of tap water from Rochester, lower body radium content among samples from the Northwest than for samples from the Rochester area would be expected. The fact that the tap water values differ by a factor of at least 30 between Rochester and the Northwest, while radium content of cadavers from the two areas differ by a factor of only two and a half, is not too surprising. Shadley (4) in his work on the radium content of common foods concludes that much more

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Progress Report

ABSORPTION OF PLUTONIUM THROUGH THE LIVING SKIN OF THE RAT

M. H. Weeks and W. D. Oakley

Plutonium applied as a 10 N HNO₃ solution is absorbed over a period of five days to the extent of about two per cent of the total applied. This percentage absorption is not greatly affected by changes in plutonium concentration of the contaminating solution over a range of 10⁴. At lower solution acidities the percentage absorbed decreases, probably due to greater mechanical loss of plutonium from the site of contamination.

The results of preliminary studies on percutaneous absorption of plutonium were reported in the previous annual report (1), and in HW-30232 (2). In brief, these studies indicated that the amount of plutonium passing into the carcasses of rats depended upon the normality of the acid vehicle, the area contaminated, and the length of time exposed; the percentage of the applied plutonium absorbed increasing with greater acidity, larger area, and longer periods of exposure. These investigations were continued in an attempt to more clearly define the parameters controlling the percutaneous absorption of plutonium. Results of two additional experiments are presented.

METHODS

The extent of absorption of plutonium through the skin of rats was studied in an experiment where all experimental factors were held constant except the concentration of plutonium in the solution applied. Ten microliters of a solution of Pu(IV) in 10 N HNO₃ was applied to a 1 cm² skin area. Five groups of five animals each were exposed for five days to plutonium solutions ranging in concentration from approximately 0.015 μg

to 146 μg . At the end of the exposure period, animals were sacrificed and the plutonium content of the contaminated segment of skin, and of the remaining total carcass (gastro-intestinal tract discarded) determined.

The influence of acidity on absorption was examined in an experiment in which plutonium was applied as a 0.1 N, 2.0 N, or 10.0 N HNO_3 solution. Three groups of five animals each were exposed for five days to 10 μl of solution containing approximately 1 μg Pu(IV) spread over a 1 cm^2 area of skin. Animals were sacrificed and analyzed as described for the previous experiment.

RESULTS AND DISCUSSION

The results of the experiment on plutonium concentration are shown in Table 1. Differences in per cent absorbed at the different concentration levels are not statistically significant, except for the case of the lowest concentration employed. The low recovery of plutonium from the contaminated area of skin is not surprising, considering the long period of exposure. Any plutonium lost from the skin and ingested by the animal would not significantly affect results, since the gastro-intestinal tract was excluded from the analyses, and absorption from the tract would be insignificant compared with absorption through the skin (3).

Results of the experiment on acidity of the contaminating solution are shown in Table 2. The percentage of applied plutonium which was absorbed increased with increasing acidity. It should be noted, however, that recovery of plutonium from the contaminated skin area was much lower at the lower acidities, and it seems quite probable that the apparent decrease in absorption at lower acidities is due to mechanical loss of plutonium from the contaminated area before absorption can occur. Higher acidities result in skin damage which immobilizes a greater proportion of the applied plutonium at the exposure site, but does not necessarily increase the rate of absorption.

TABLE 1

Effect of Plutonium Concentration on Percutaneous Absorption
(Five-day exposure to 1 cm^2 skin area, 10 N HNO_3 solution of Pu(IV) nitrate)

Pu Applied (d/m)	Per Cent of Applied Pu Recovered from Skin (avg. \pm std. dev.)	Per Cent of Applied Pu Absorbed (avg. \pm std. dev.)
1.6×10^7	41 ± 6	2.2 ± 1.0
1.7×10^6	48 ± 4	1.2 ± 0.3
1.1×10^5	46 ± 4	1.8 ± 0.5
1.8×10^4	37 ± 7	2.2 ± 1.0
1.9×10^3	46 ± 14	3.1 ± 1.0

TABLE 2

Effect of Acidity of Contaminating Solution on Percutaneous
Absorption of Plutonium
(Five-day exposure to 1 cm^2 skin area, $1 \mu\text{g}$ Pu(IV) nitrate)

Normality of HNO_3 Solution	Per Cent of Applied Pu Recovered from Skin (avg. \pm std. dev.)	Per Cent of Applied Pu Absorbed (avg. \pm std. dev.)
0.1	10 ± 4	0.29 ± 0.23
2.0	13 ± 2	0.43 ± 0.16
10.0	54 ± 5	1.6 ± 0.15

ACKNOWLEDGMENTS

We wish to acknowledge the technical assistance of Joan O. Hess and Elizabeth D. Cline in this work.

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Abstract

ABSORPTION OF PLUTONIUM FROM THE GASTRO-INTESTINAL
TRACT OF RATS: EFFECT OF PLUTONIUM CONCENTRATION

J. Katz* and M. H. Weeks

Previous studies (1, 2) of plutonium absorption from solutions whose concentration approximated the maximum permissible concentration (MPC) of plutonium in aqueous media**, have now been extended to include studies made at concentration levels varying from 10 to 50,000 times MPC. A detailed account of these studies appears in HW-30220 (3).

Seven groups of 20 rats each received 124 one ml doses of plutonium by stomach tube. Plutonium mass concentrations were approximately 10; 100; 500; 5,000; 10,000; 20,000; and 50,000 times MPC. All groups received Pu²³⁹ except the 10 x MPC group which received Pu²³⁸. The animals were sacrificed and analyzed as previously described (1, 2). Results of the present study combined with the previous data obtained at lower plutonium concentrations are shown in Table 1.

The results indicate no significant effect of plutonium concentration on absorption. The data are quite consistent, except for the 100 x MPC and 500 x MPC groups. In view of the large confidence limits associated with the averages of these groups, the values are not unreasonable in a probability sense. These groups were fed the lowest concentration levels of Pu²³⁹ employed and analyses were consequently less accurate than in the case of the other groups.

The average absorption and retention is nearly 40 times lower than the figure of 0.1 per cent absorption assumed in the calculation of the presently established MPC.

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** MPC of Pu²³⁹ in aqueous media = 1.5×10^{-6} $\mu\text{c/ml}$, or 2.4×10^{-5} $\mu\text{g/ml}$ (4).

TABLE 1

Average Per Cent of Gastro-Intestinal Absorption and Deposition
of Plutonium Fed to Rats
(95 Per Cent Confidence Limits)

Concentration Fed (x MPC)	Absorption and Deposition (Per Cent of Amount Fed)		
	Skeleton	Soft Tissue	Total
0.12	.0019 ± .0005	.0001 ± .0003	.0020 ± .0006
0.40	.0025 ± .0003	.0004 ± .0004	.0029 ± .0005
1.2	.0022 ± .0002	.0002 ± .0001	.0024 ± .0004
10	.0022 ± .0005	.0000 ± .0013	.0022 ± .0013
100	.0000 ± .0069	.0000 ± .024	.0000 ± .025
500	.0011 ± .0017	.0004 ± .0047	.0015 ± .0051
5,000	.0028 ± .0004	.0002 ± .0004	.0030 ± .0007
10,000	.0028 ± .0004	.0004 ± .0003	.0032 ± .0004
20,000	.0024 ± .0004	.0002 ± .0001	.0026 ± .0004
50,000	.0028 ± .0004	.0005 ± .0001	.0033 ± .0004
Pooled Average	.0025 ± .0003	.0003 ± .0007	.0028 ± .0008

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Progress Report

INFLUENCE OF PLUTONIUM CONCENTRATION
ON EFFECTIVENESS OF THERAPEUTIC AGENTS

M. H. Weeks and W. D. Oakley

The effectiveness of zirconium citrate and CaEDTA in preventing the retention of plutonium in rats was shown to be not significantly influenced by the amount of plutonium administered to the animals.

All previous tests of therapeutic agents for preventing the deposition and retention of plutonium have been made on animals administered relatively large amounts of plutonium. It was considered essential to determine whether the much smaller amounts of plutonium which would still be considered hazardous in a human were removed with the same efficiency as the larger amounts.

METHODS

Three groups of six Sprague-Dawley, male rats were injected intraperitoneally with one ml of a solution containing 0.0014 μg Pu(IV) citrate, pH 6. Plutonium-238 was employed in order to obtain adequate counting rates. One group served as a control, the second group received, intraperitoneally, 4 ml of zirconium citrate solution containing 50 mg zirconium, and the third group received, intraperitoneally, 3 ml of a solution containing 600 mg of calcium disodium ethylenediaminetetraacetic acid (CaEDTA). Therapeutic agents were administered 30 minutes following plutonium injections. Animals were sacrificed after 30 days and plutonium determined in the separated skeleton and soft tissues.

RESULTS AND DISCUSSION

Results of the plutonium analyses are shown in Table 1. Also included in Table 1 are similar data from two previous experiments in which a 10^5 times larger amount of plutonium was administered. Comparison of the results at the two plutonium levels indicates no significant differences in therapeutic effectiveness attributable to plutonium concentration.

The mass concentration of Pu^{238} present in the animals employed in this experiment is approximately equivalent to the mass concentration of Pu^{239} in humans which is presently accepted as the maximum permissible level. It can, therefore, be concluded that data previously obtained by many workers at relatively high plutonium concentrations are also applicable to the practical situations most likely to be encountered in incidents of human plutonium contamination.

ACKNOWLEDGMENTS

We wish to acknowledge the technical assistance of Joan O. Hess and Elizabeth D. Cline.

TABLE 1

Influence of Plutonium Concentration on Effectiveness of Therapeutic Agents

	Per Cent of Administered Pu Deposited*		
	1.4 m μ g Pu ²³⁸ Administered	138 μ g Pu ²³⁹ Administered	141 μ g Pu ²³⁹ Administered
Control			
Soft tissue	15 \pm 4	20 \pm 2	25 \pm 5
Skeleton	58 \pm 4	45 \pm 5	46 \pm 5
Total animal	73 \pm 3	65 \pm 7	71 \pm 5
Zr Citrate Treated			
Soft tissue	15 \pm 4	21 \pm 6	
Skeleton	13 \pm 1	14 \pm 6	
Total animal	28 \pm 4	35 \pm 11	
CaEDTA Treated			
Soft tissue	5 \pm 1	7 \pm 1	11 \pm 5
Skeleton	33 \pm 3	33 \pm 4	27 \pm 3
Total animal	38 \pm 3	40 \pm 4	38 \pm 3

* Each entry is the average of analyses on four to six animals \pm 68.2 per cent confidence limits.

Abstract

RELATIVE EFFECTIVENESS OF VARIOUS AGENTS FOR PREVENTING
THE INTERNAL DEPOSITION OF PLUTONIUM IN THE RAT

J. Katz*, M. H. Weeks and W. D. Oakley

The relative therapeutic effectiveness of zirconium citrate, zirconium malate, and disodium calcium ethylenediaminetetraacetic acid (CaEDTA) in removing plutonium from the rat was compared, following single or repeated, and early or late injections of these agents. By administering these substances separately and in combination with each other and then analyzing the plutonium distribution in total soft tissue and skeleton, it was hoped to determine whether there existed a synergism among these therapeutic agents. Skeletons of the rats in the early therapy studies were subdivided into skull, vertebrae, ribs, and limbs and girdle segments in order to determine whether certain bones exhibited a special affinity for plutonium and whether this avidity was modified by the various therapeutic agents. Details of the experimental procedures and the results obtained are reported in HW-30231 (1).

Briefly, the results indicated that prompt administration of CaEDTA was the most effective procedure for reducing soft tissue retention of plutonium. Prompt administration of zirconium citrate was the most effective procedure for preventing deposition of plutonium in the skeleton. Simultaneous administration of CaEDTA and zirconium citrate seemed to combine the good effects of both and was the most effective procedure employed, resulting in a total retention of approximately 21 per cent of the administered plutonium, compared with approximately 75 per cent retention in the untreated controls.

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Zirconium malate was generally comparable to zirconium citrate in its ability to prevent skeletal deposition of plutonium, but showed a marked tendency to increase soft tissue retention and appeared to be more toxic than zirconium citrate. Repeated injections of the therapeutic agents offered no significant advantage over single dosage. All forms of therapy initiated 30 days after plutonium administration were ineffective in removing the plutonium fixed in the body of the rat. All skeletal segments exhibited a comparable affinity for plutonium with the exception of the bones of the skull which showed a lower activity density.

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Progress Report

COMPARISON OF CaEDTA WITH ZIRCONIUM CITRATE IN
PROMOTING EXCRETION OF PLUTONIUM FROM THE DOG

R. W. Wager and L. A. Temple

Single and multiple intravenous injections of zirconium citrate or CaEDTA at several dose levels were administered to dogs previously injected with plutonium. Under the experimental conditions outlined, removal of plutonium, as determined by analyses of urine, was increased most by repeated injections of zirconium citrate. Influences of the treatments on distribution of plutonium in tissues are shown.

The use of zirconium to promote the excretion of administered plutonium was first described by Schubert in 1947 (1). Foreman (2) first described the utilization of calcium sodium ethylenediaminetetraacetate for the same purpose. In a preliminary report from this laboratory the value of zirconium citrate in the removal of Pu (IV and VI) in the dog was described (3). The present paper reports a series of similar experiments designed 1) to determine the optimal dose and most efficacious mode of zirconium citrate administration, and 2) to compare the efficacy of CaEDTA with zirconium citrate.

METHODS

Mongrel female dogs ranging in weight from 6 to 16 kg were used. Each dog was housed in a metabolism cage designed to separate urine and feces which were collected daily. The diet of the experimental dogs was approximately one pound of horse meat mixed with dog checkers (Purina). Water was available at all times.

After the dogs were anesthetized with "Nembutal" (Pentobarbital Sodium, Abbot - 25 mg/kg i. v.), an incision was made, under aseptic conditions, exposing one of the external jugular veins. A hypodermic needle (#13) of a diameter sufficient to pass a polyethylene catheter (o. d. 1.70 mm, i. d. 1.17 mm) was introduced into the vein, and the catheter threaded through the needle until the end was in the precaval region. Blood could be aspirated through the catheter with ease. The lumen of the catheter was then filled with a heparin solution (10 U. S. P. units/ml), the needle removed from the vein, and the free end of the catheter stoppered with a glass plug. Firm ties fixed the catheter in place in the vein, and the incision was closed with the free end of the catheter to the exterior. The lumen of the catheter was filled with a heparin solution when not in use.

A glass catheter was introduced into the bladder and allowed to remain in place during the time the dog was under anesthesia. Urine was collected and discarded just prior to plutonium injection and the bladder rinsed with 50 ml of a saturated boric acid solution. This in-dwelling catheter allowed urine samples to be taken at will, the bladder being flushed after each sampling. Complete urinary collection was obtained for each time interval.

A plutonium citrate solution (Pu IV) was administered i. v. (6.4 $\mu\text{c}/\text{kg}$) to each dog. The solution of plutonium citrate first was drawn into a syringe that had been treated with "Dri-Film" (Dimethyldichlorosilone), and a three-way valve attached. The solution was then injected into the jugular vein opposite the one containing the polyethylene catheter. To minimize the hazard of contamination, the three-way valve was used to flush the apparatus with isotonic saline solution from an attached container. At 5, 10, 20, 40, and 60 minutes after plutonium citrate was administered, a blood sample was aspirated through the polyethylene catheter and placed in a "Dri-Filmed" heparinized tube for subsequent radioassay.

One hour after the administration of the plutonium citrate solution, all urine was removed and the bladder was flushed. Another blood sample was then aspirated via the polyethylene catheter, and treatment was initiated.

The zirconium citrate solution used in these experiments was a sterile, colorless solution with a pH of about 6.3. It contained 25 mg of zirconium per ml, and sodium ion and citric acid concentrations equivalent to a 10 per cent sodium citrate solution. The CaEDTA used was a 20 per cent solution (Riker Laboratories, Inc.). The different doses of zirconium citrate were made to be the volume of the single 100 mg/kg dose (4 ml/kg) by the addition of a 135 mg/ml sodium chloride solution. The amount of sodium ion in each dose for all zirconium treated animals was thus made identical so as to minimize differences in urine volume attributed to administered saline. Whenever CaEDTA was administered, volumes were made equal to that of zirconium citrate by addition of the saline solution. The dogs which received multiple treatments received only the first treatment while under anesthesia. Subsequent injections were made through the indwelling polyethylene catheter. Some of the dogs received zirconium citrate solution containing added calcium and magnesium ions in the proportions of 1 mol calcium to 1.64 mol magnesium so as to mitigate the hypocalcemic effect of administered citrate.

One hour after treatment was initiated, a blood sample was aspirated and a urine sample was taken; this sampling routine was repeated two hours later. At the end of this routine the glass urinary catheter was removed and the dogs were placed in metabolism cages. Urine and fecal samples were collected daily, and a blood sample was aspirated through the polyethylene catheter on days one, three, five, and ten. On the tenth day the dogs were sacrificed, and aliquots of lung, vertebrae, spleen, rib, liver, kidney, femur and skeletal muscle were taken from all animals for subsequent radioassay.

The treatments applied and results obtained are summarized in Table 1.

TABLE 1
Effects of I. V. Injections of Zirconium Citrate and CaEDTA on Plutonium Excretion from Dogs

Agent	Administered (mg/kg)*	Frequency of Treatment	No. of Dogs	Per Cent of Pu Excreted During 10 Days	Amount of Pu in tissues 10 days after initial injection of 6.4 $\mu\text{c}/\text{kg}$		
					Spleen ($\mu\text{c} \times 10^2$)	Liver ($\mu\text{c} \times 10^2$)	Vertebrae ($\mu\text{c}/\text{g} \times 10^2$)
None	(Control)	---	2	2	14	600	4.8
Zr. citrate	12.5	Single	1	13	56	1100	2.6
Zr. citrate	25	Single	3	67 \pm 17	30	600	0.8
Zr. citrate	50	Single	5	70 \pm 21	38	1200	0.6
Zr. citrate	100	Single	2	82 \pm 40	30	550	0.9
Zr. citrate	50	1/day for 5 days	2	95 \pm 10	14	360	0.8
CaEDTA	73	Single	1	1	19	1200	2.4
CaEDTA	147	Single	1	15	95	1900	2.6
CaEDTA	37	2/day for 5 days	2	29 \pm 2	16	1200	1.6
CaEDTA	73	1/day for 5 days	2	19 \pm 5	12	500	2.8
CaEDTA	147	1/day for 5 days	1	43	32	1500	7.5
CaEDTA	73	2/day for 5 days	1	63	15	1000	3.1

* Expressed as milligrams of zirconium or CaEDTA per kilogram of dog.

RESULTS AND DISCUSSION

In the zirconium citrate treated dogs and when the higher dose of CaEDTA was applied, the per cent plutonium excreted via the feces is small when compared to the urinary excretion, as can be seen in Table 2. In the control dogs the amount of plutonium excreted in the urine and feces is in the expected ratio (2), and corresponds to previous findings in this laboratory. In the treated dogs the fecal volume is often decreased because of the anorexia produced. The significance of the decreased fecal excretion of plutonium in some of the treated animals is therefore somewhat obscured.

TABLE 2

Comparison of Per Cent of Total Dose of Pu Excreted in Ten Days
in Urine and Feces of Animals of One Series when Treated
with Zirconium Citrate or CaEDTA

	Control	Zirconium Citrate				CaEDTA	
		12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg	73 mg/kg	147 mg/kg
Urine	1.8	12	53	60	48	1.4	14
Feces	3.0	1.5	2.6	1.8	0.4	2.6	.6

Table 1 indicates that multiple treatments with zirconium citrate (50 mg/kg) are as effective as the single 100 mg/kg dose reported in previous findings from this laboratory (2). In analyzing tissues of the sacrificed experimental dogs, liver and bone are found to contain the largest amount of plutonium, and the concentration of plutonium in vertebrae from dogs treated with zirconium citrate is significantly lower than that from

dogs treated with CaEDTA. However, the different treatments did not cause a significant difference in the concentration of plutonium in liver. The plutonium content of other tissues did not indicate obvious correlation with treatment.

Figure 1 is chosen to represent what appears to be conditions for greatest efficiencies of plutonium removal from dogs treated with the two agents. This, of course, should not be taken to represent the average case. Table 1 indicates that multiple treatments with 73 mg/kg CaEDTA result in elimination of only 63 per cent of the administered plutonium, or only about two-thirds of the observed excretion due to multiple application of 50 mg/kg zirconium citrate. Although the rate of urinary excretion of plutonium is increased following CaEDTA, at no time is the effect as marked as that observed following zirconium citrate. For instance, not evident in Figure 1 is the fact that three hours after the application of zirconium citrate, 35 per cent of the administered plutonium appeared in the urine, whereas only 12 per cent of administered plutonium was present in urine three hours after CaEDTA administration.

ACKNOWLEDGMENTS

The authors wish to acknowledge the very capable assistance of C. H. Hemphill in preparing samples for radioassay, and G. E. Pilcher and D. W. Gaylor for compiling statistical data.

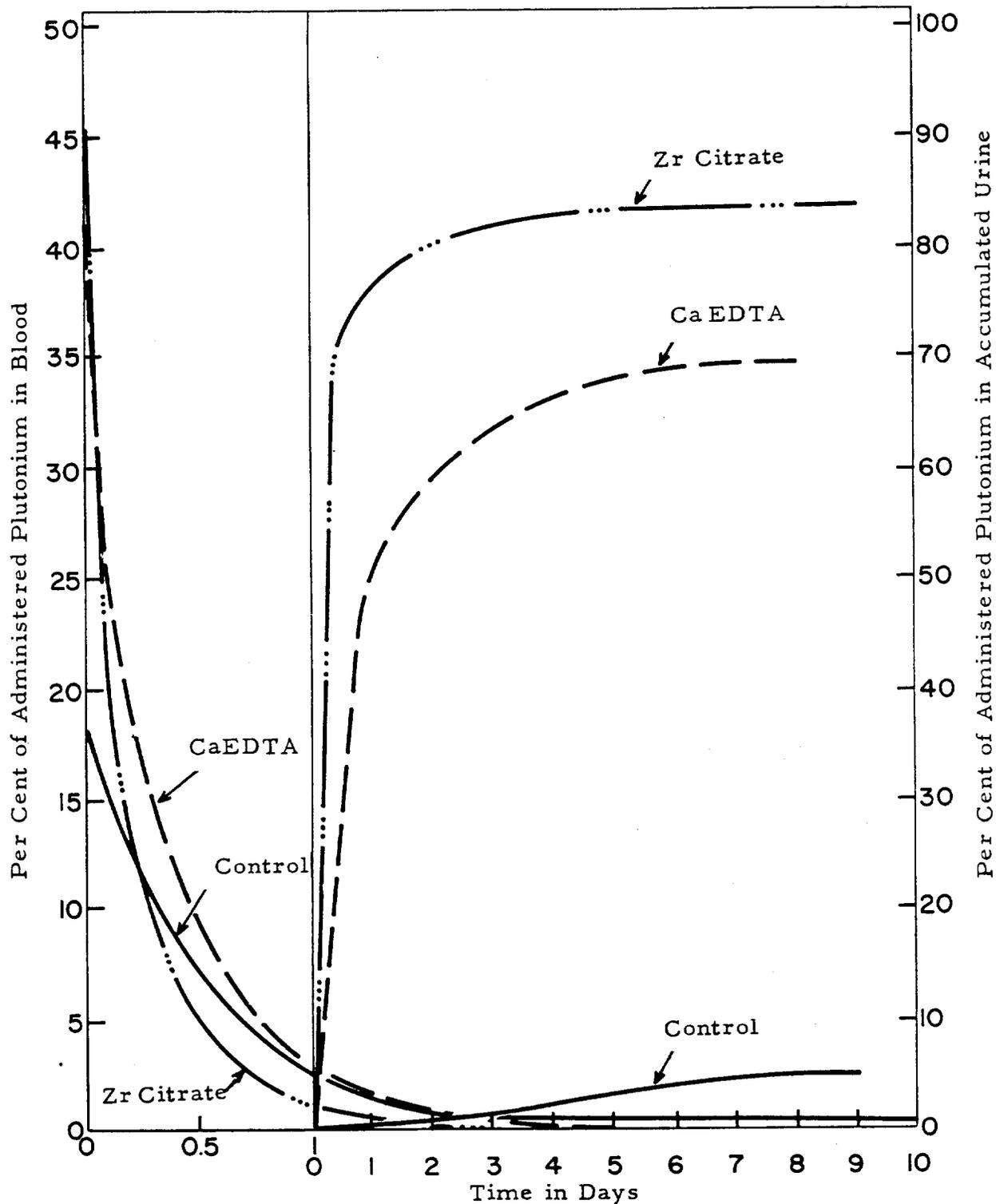


FIGURE 1. EXCRETION OF PLUTONIUM FROM THREE DOGS INJECTED WITH PLUTONIUM. CONTROL DOG RECEIVED NO TREATMENT. ZIRCONIUM CITRATE TREATED DOG RECEIVED ONE DOSE/DAY FOR FIVE DAYS. CaEDTA TREATED DOG RECEIVED TWO DOSES/DAY FOR FIVE DAYS. (SEE TEXT)

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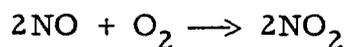
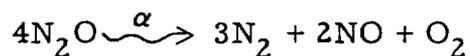
DETERMINATION OF ALPHA PARTICLE EMITTERS IN THE LUNG

D. R. Kalkwarf and H. A. Kornberg

The alpha-particle induced decomposition of nitrous oxide was investigated as a means of detecting isotopes emitting this type of radiation when deposited in the lung. The sensitivity was found to be too low for detecting Pu²³⁹ in microgram amounts under reasonable conditions.

Due to the short range of alpha particles, isotopes emitting this type of radiation and residing in the lung are particularly difficult to detect. As an approach to this problem, it was felt that a gaseous chemical reaction which was induced by alpha particles could be used to advantage. In this case, the reactants would be brought into intimate contact with the entire lung surface; and the products, whose amounts would be a measure of the amount of radioisotope present, could be continually removed by ordinary breathing and collected for analysis.

The reaction which has been investigated thus far is the alpha particle induced decomposition of nitrous oxide. Balanced equations for the reactions involved are:



The use of nitrous oxide is of particular advantage since it is a non-irritating gas whose physiological effects are well known, and the product gas, nitrogen dioxide, would not be present in harmful concentrations. The purpose of the present investigation was to determine how much energy as ionizing radiation is required to cause the formation of a detectable amount of NO₂ by these reactions and also if the yield could be increased by the presence of other gases.

METHODS

The source of alpha particles used was a deposit of $\text{Pu}^{239}\text{O}_2$ upon a platinum disc. The activity of the source corresponded to 18 μg of Pu^{239} and the rate of energy emission as alpha particles to the surrounding gas was calculated to be 6.5×10^{12} ev/min. A glass reaction vessel with a radius slightly greater than the range of these alpha particles was used with the source mounted in the center, and nitrous oxide alone and in mixture with other gases was admitted for definite time periods.

The NO_2 formed during the irradiation was used as a measure of the extent of N_2O decomposition, and was determined using a modification of the method described by Rider and Mellon (1) for the analysis of nitrate ion. The contents of the reaction flask were transferred to a flask containing 5 ml of sulfanilic acid solution and the NO_2 gas was allowed to dissolve and diazotize the sulfanilic acid. The remainder of the procedure followed that in the above reference except on a smaller scale, and the final colored solution was examined in a spectrophotometer at 520 $\text{m}\mu$. Using this method, as little as 4×10^{14} molecules of NO_2 could be detected.

RESULTS AND DISCUSSION

None of the gas mixtures used produced a significantly greater yield than could be obtained with pure N_2O , although small amounts of CO_2 appeared to lower it. Using pure N_2O , it would take approximately one day for a detectable amount of NO_2 to accumulate with the source used. Thus this reaction does not appear suitable as a means of determining microgram amounts of plutonium in the lung.

TABLE I
Yield of NO₂ from the Alpha-Particle Induced Decomposition of N₂O
(30.0°C, 1 atm. total pressure)

Run	Mole Fraction of Reactants				Irradiation Time (Minutes x 10 ⁻³)	Yield (Molecules of NO ₂ per 100 ev)
	N ₂ O	N ₂	CO ₂	O ₂		
N ₂ O III	1.00				213	11
N ₂ O V	1.00				1.0	13
N ₂ O VI	1.00				1.0	9
N ₂ O VII	1.00				1.1	12
N ₂ O IX	1.00				1.6	10
N ₂ O-O ₂ I	0.80			0.20	1.2	15
N ₂ O-O ₂ II	0.81			0.19	3.9	10
N ₂ O-O ₂ III	0.81			0.19	1.3	17
N ₂ O-O ₂ IV	0.20			0.80	1.6	10
N ₂ O-N ₂ I	0.79	0.21			2.3	6
N ₂ O-N ₂ II	0.51	0.49			2.9	6
N ₂ O-CO ₂ I	0.93		0.07		4.3	2
N ₂ O-CO ₂ II	0.94		0.06		3.7	4
N ₂ O-H ₂ O I	0.97				4.2	4
N ₂ O-O ₂ -H ₂ O I	0.74			0.23	1.1	14
N ₂ O-O ₂ -H ₂ O II	0.52			0.45	2.2	8
N ₂ O-O ₂ -CO ₂ I	0.78		0.06	0.16	2.8	2
				0.03		
				0.03		
				0.03		

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Progress Report

BETA IRRADIATION OF THE SKIN OF SHEEP

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

Methods are described that were employed in irradiating sheep with beta-particle emitting plaques in an attempt to develop the pathological syndrome of beta irradiation in the skin of sheep.

The exact nature of beta irradiation of the skin of sheep is unknown. This paper is a preliminary report of experiments designed to determine the threshold level of beta irradiation that is injurious to the skin of sheep and the pathological syndrome of such irradiation.

METHODS

The dorsal and lateral surfaces of purebred Suffolk sheep were sheared with electric sheep shears, brushed clean of dirt, and sheared again with surgical clippers one day prior to irradiating with a beta-particle emitting plaque. Particular care was taken to avoid any mechanical trauma to the skin during this procedure. Approximately 0.01 gram of wool per square centimeter remained on the surface of the skin following the above procedure. A P^{32} plaque manufactured and calibrated by Oak Ridge National Laboratories and delivering approximately 6800 rad/hour was used in the initial phase of the experiment. Manipulation and application of the plaque to the skin surface was facilitated by the use of a locally designed plaque container and applicator (Figures 1 and 2). The initial phase of the experiment involved three sheep, each irradiated at four different sites on each side of the dorsal lateral surface of the back. The various sites received dose levels of either 2000, 4000 or 8000 rads per site. As much variation as possible was employed in distributing the various dose levels among the restricted number of sites.

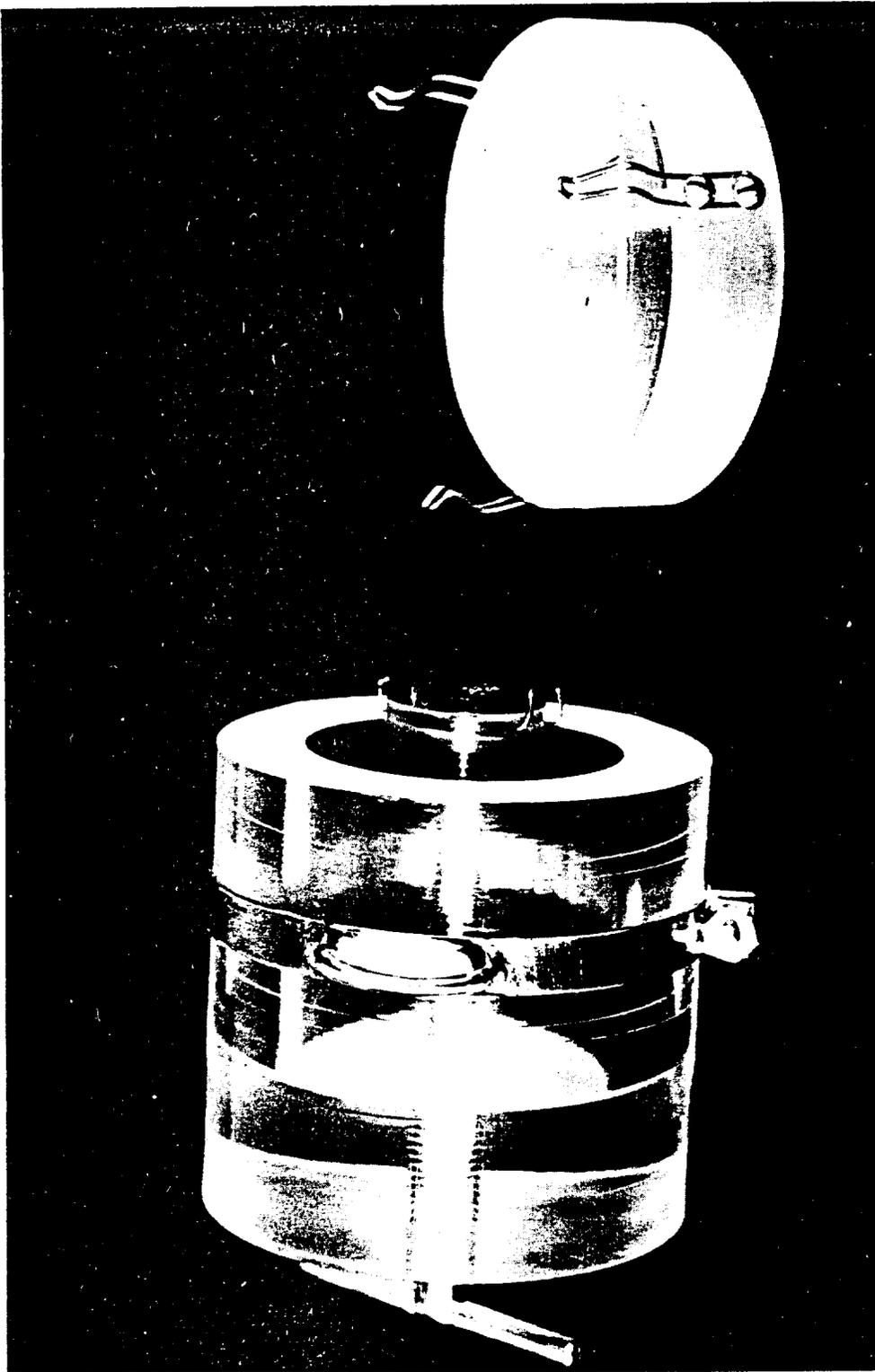


FIGURE 1. LOCALLY FABRICATED BETA PLAQUE APPLICATOR THAT FACILITATES SAFE HANDLING AND EFFICIENT APPLICATION.

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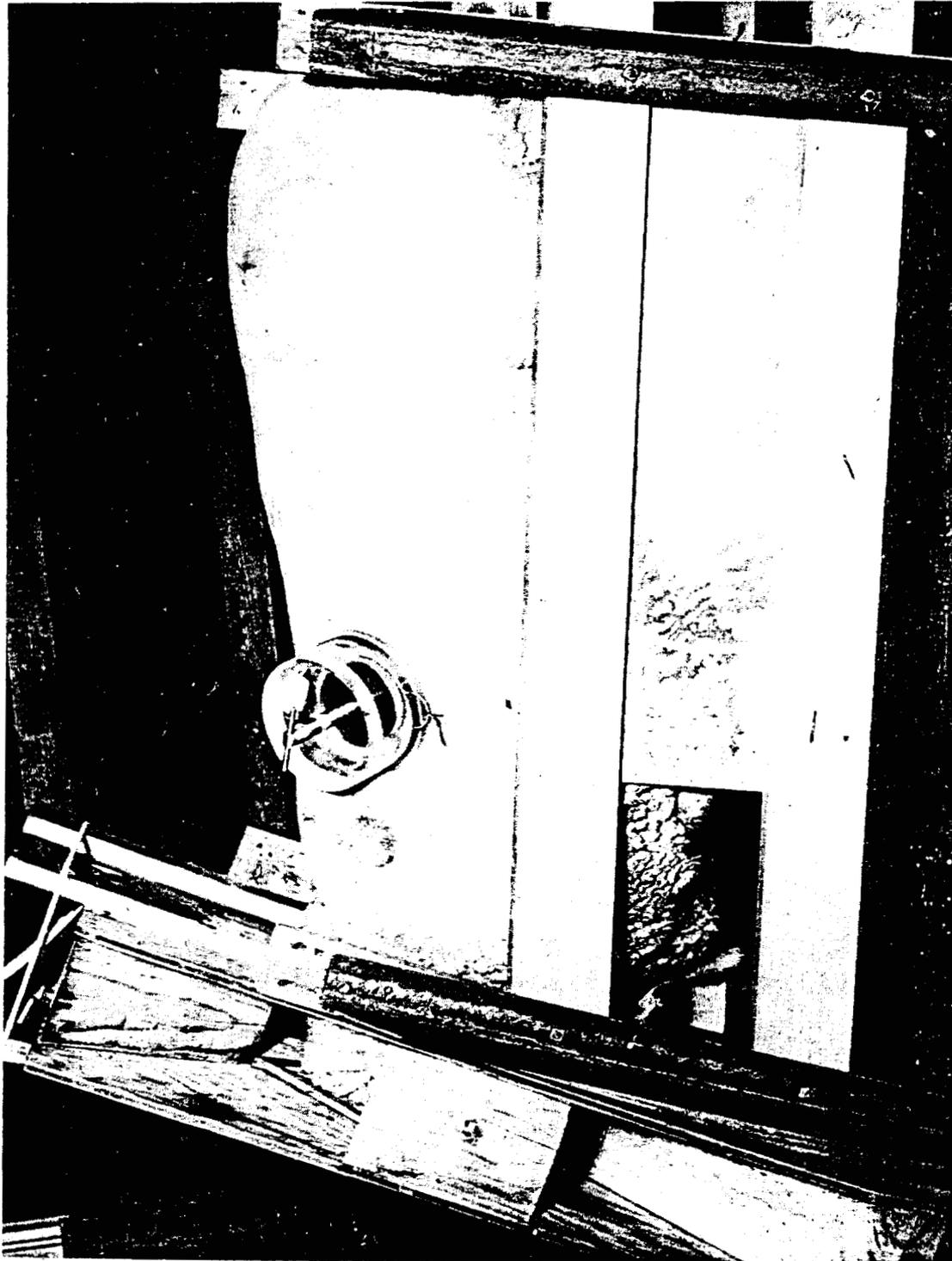


FIGURE 2. BETA PLAQUE (P^{32}) APPLICATOR IN POSITION ON EXPERIMENTAL SHEEP.

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Two irradiated sites on each animal remained as control sites. Biopsies were removed from the remaining six sites at definite time intervals (i. e., 6, 12, 24, and 48 hours post irradiation, followed by 4, 7, 12 and 19-day intervals, and at weekly intervals thereafter for approximately 10 to 12 weeks). The remaining two irradiated control areas were not submitted for biopsy. Biopsy sections were fixed in Bouin's fixative and 10 per cent formalin. Histology sections were stained with hematoxylin and eosin. Weights were taken weekly and blood samples drawn twice weekly initially and at weekly intervals thereafter for determination of leucocyte and differential cell counts.

In an attempt to evaluate the significance of the different energy levels delivered by different isotopes, a second phase of this experiment involves the use of a Sr^{90} source also in the form of a plaque. In using this source an attempt will be made to duplicate as nearly as possible the procedures employed in irradiating with the P^{32} plaque. Preliminary studies involved the use of a locally prepared Sr^{90} plaque on one sheep, applying dosages of from about 10,000 to over 50,000 rads.

RESULTS AND DISCUSSION

Preliminary results applying phosphorus plaques indicate hyperesthesia of the skin and swelling at the 8000 rad dose only. No lesions are grossly visible at 10 days except a slight discoloration over the irradiated area. The preliminary observations applying the Sr^{90} plaque indicated an extreme hyperesthesia of the skin. Hair loss was evident over the entire irradiated area and had not begun re-growth at two months following irradiation.

Biopsied sections from these areas revealed extreme pathological changes at all levels of Sr^{90} plaque application. It must be indicated that these observations are very preliminary and the dosages applied using Sr^{90} are only approximations.

ACKNOWLEDGMENTS

The assistance of D. E. Warner and D. W. Norgard of the Biology Control Unit and W. E. Roesch of the Biophysics Section in the preparation and calibration of the Sr⁹⁰ plaque and the aid of the Radiological Monitoring Unit in applying the plaques are gratefully acknowledged.

Progress Report

TOXICITY OF I¹³¹ IN SHEEPX. LOW-LEVEL CHRONIC EFFECTS

L. K. Bustad, L. A. George, C. M. Barnes, S. Marks,
D. E. Warner, and H. A. Kornberg

This report summarizes the recent findings in a continuing experiment designed to determine the minimal level of I¹³¹ that may be toxic when administered chronically to sheep. Toxicity was shown in the 5 $\mu\text{c}/\text{day}$, 15 $\mu\text{c}/\text{day}$ and 30 $\mu\text{c}/\text{day}$ levels. Two new levels were initiated at 0.5 and 1.5 $\mu\text{c}/\text{day}$ when there were definite indications of slight toxicity at the 5 $\mu\text{c}/\text{day}$ level with time.

This paper represents a progress report of a continuing experiment whose primary objective is to determine the maximal level of I¹³¹ administered daily during the entire lifetime of sheep which will result in no toxic manifestations. Papers I to IV of this series summarized results obtained during the first twenty months of the investigation. Manifestations of toxicity during that period were limited to sheep fed 240 $\mu\text{c}/\text{day}$ while no evidence of damage was observed in the test animals fed 5 μc or less per day (1). Papers V through IX summarized the general observations made during 1952 on the original experimental animals, their offspring and new groups which were established at levels of 15, 45, and 135 $\mu\text{c}/\text{day}$ (2). The present report summarizes the general observations made during 1953 on those original animals still remaining, their offspring and on new groups of sheep established at 0.5, 1.5, and 30 $\mu\text{c}/\text{day}$ levels.

METHODS

The number of animals in groups under experimentation in January, 1953, were as follows:

TABLE 1

Number of Animals in Groups at 32 Months

$\mu\text{c I}^{131}$ Fed/Day	30	15	5	0.15	Control	
					Project	Off-Project
No. of Original Ewes			9	10	10	9
1950 Offspring (Ewes)		3	6	6	6	7
1951 Offspring (Ewes)	6	0	5	6	6	6
1952 Offspring (Ewes)		4	8	7	9	6

The remaining ewes in the group fed 240 $\mu\text{c/day}$ for 450 days were sacrificed during the year. Some of the original ewes in the control, 0.15 $\mu\text{c/day}$ and 5 $\mu\text{c/day}$ groups were also sacrificed as they approached six years of age in order to determine fetal thyroid weight and I^{131} uptake at varying stages of gestation. All animals in the 15 $\mu\text{c/day}$ and 30 $\mu\text{c/day}$ groups were sacrificed 22 and 7 months respectively after the beginning of I^{131} administration when thyroid damage was observed in their offspring. New groups were then initiated at levels of 0.5 and 1.5 $\mu\text{c/day}$, providing the following breakdown of the experimental groups:

TABLE 2

Number of Animals in Groups at 40 Months (August 1953)

$\mu\text{c I}^{131}$ Fed/Day	5	1.5	0.5	0.15	Control	
					Project	Off-Project
No. of Original Ewes	8			8	9	7
1950 Offspring	6			6	6	5
1951 and 1952 Offspring	4	6	6	8	6	2
1953 Offspring	9			4	9	8

Necropsies were also performed on representative numbers of lambs at six, eight, and nine months of age and yearlings from the 5 $\mu\text{c}/\text{day}$ group. Weekly monitoring of all sheep thyroids continued. The monitoring instruments used were previously described (3). Blood samples were collected from all ewes every three weeks. Hematological procedures included total white blood cell count, differential count and packed cell volume determination. Blood volume was determined by the Evans Blue dye method. A 0.5 per cent aqueous solution of the dye was injected into a polyethylene catheter, which was introduced 24 hours previously into the external jugular vein of a sheep confined in a stall. Following injection and between sampling, the catheter was filled with a heparin solution (1:1000) and taped to the back of the animal. This procedure facilitated withdrawing blood samples without restraining or exciting the test animal.

Blood chemistry procedures included creatinine and inorganic phosphorus determination. In addition, specimens of blood, urine and feces were submitted every four weeks for assay of radioactivity. Routine sampling of tissues for radioactivity and histological study was performed at necropsy on all exposed animals and on a representative number of lambs from all groups at birth and at weaning. Whole milk samples were also analyzed for I^{131} content as were the cream and skim milk fractions.

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/q) is shown in Figure 1 for the original ewes, Figure 2 for the first-year female offspring, and Figure 3 for the second-year female offspring. The Q/q of the groups fed

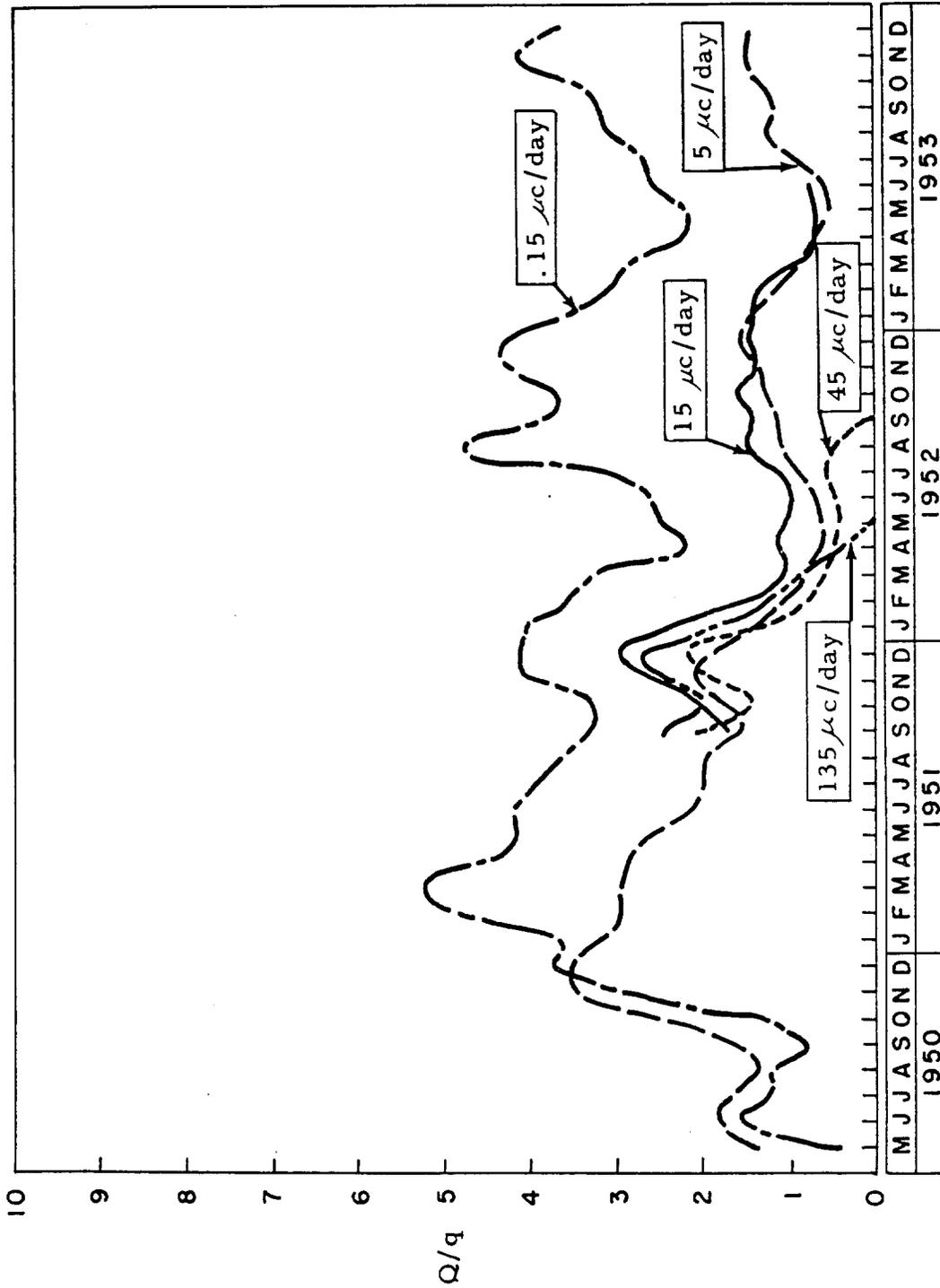


FIGURE 2
VARIATIONS OF THE RATIO Q/q (I^{131} IN THYROID/ I^{131} FED DAILY) WITH TIME
FIRST YEAR OFFSPRING

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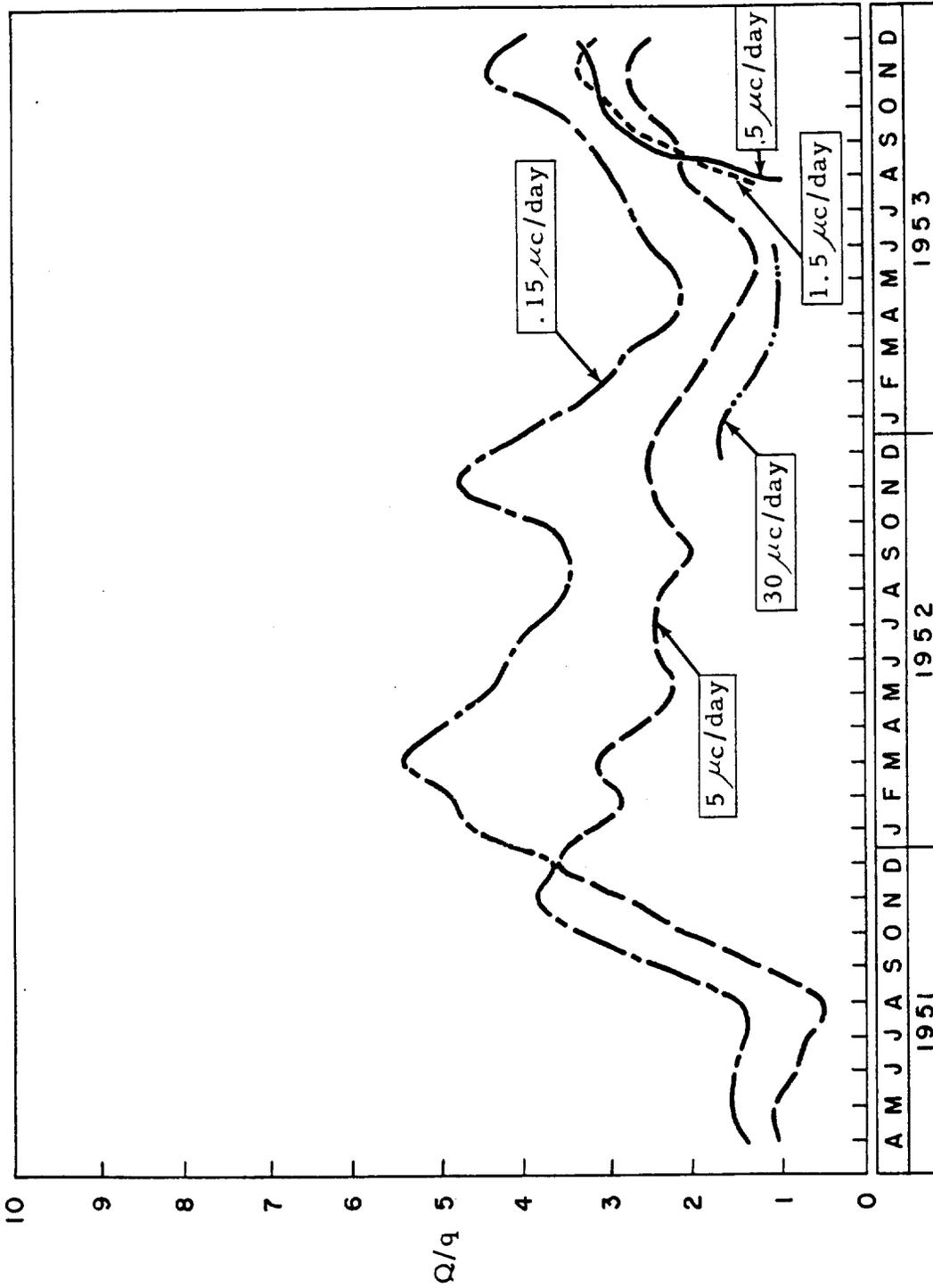


FIGURE 3
VARIATIONS OF THE RATIO Q/q (^{131}I IN THYROID/ ^{131}I FED DAILY) WITH TIME
SECOND YEAR OFFSPRING

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30 μc and 15 $\mu\text{c}/\text{day}$ had fallen to one or lower at the time of sacrifice. The lower Q/q of the 5 $\mu\text{c}/\text{day}$ groups when compared with the 0.15 $\mu\text{c}/\text{day}$ groups is most marked in the first-year offspring.

Clinical Observations

All lambs born to the ewes appeared normal at birth except two lambs who were small in size born to the ewes remaining in the 240 $\mu\text{c}/\text{day}$ group. One of the two lambs was very weak and died shortly after birth; the other lamb was sacrificed.

Necropsy and Histological Findings

Significant gross pathologic findings were observed only in animals receiving higher levels of I^{131} and were restricted to the thyroid gland. The affected animals included the four-month old lambs born to suckling ewes fed 30 $\mu\text{c}/\text{day}$ and the yearling and three-year old ewes fed 15 $\mu\text{c}/\text{day}$. The thyroid glands were reduced in size and showed an increase in content of connective tissue.

Relative to histopathological findings, the thyroid glands of three yearling ewes fed 15 $\mu\text{c}/\text{day}$ for a year showed slight damage consisting of fibrosis in each case and accompanied by edema in one instance. The offspring in this group showed distinct interfollicular edema. The administration of 30 $\mu\text{c}/\text{day}$ to six adult sheep resulted in variable damage at the end of six months. The thyroids in four animals revealed slight interfollicular fibrosis with occasional foci of inflammation and follicular degeneration. The remaining two glands were normal. Edema and inflammation were observed in the thyroids of the lambs in this group.

The histopathological observations on thyroids from animals fed 5 $\mu\text{c}/\text{day}$ for varying lengths of time are discussed in a separate report (4).

Radioiodine in Milk

TABLE 3
 Concentration of I¹³¹ in Skim Milk and Cream
 (μc/ml)

Group	Skim Milk	Cream
30 μc/day	42 x 10 ⁻⁴	11 x 10 ⁻⁴
15 μc/day	15 x 10 ⁻⁴	3 x 10 ⁻⁴
5 μc/day -		
Original Ewes	5 x 10 ⁻⁴	1.2 x 10 ⁻⁴
1st Year Offspring	8 x 10 ⁻⁴	1.7 x 10 ⁻⁴
2nd Year Offspring	6.4 x 10 ⁻⁴	1.5 x 10 ⁻⁴

The 130 samples tested showed a mean cream content of 14 per cent by volume and 10 per cent by weight. The skim milk fraction had 95 to 97 per cent of the I¹³¹ concentration although it represented only 86 per cent of the whole milk by volume. Butterfat extracted from a limited number of cream samples showed no appreciable I¹³¹ content. These data suggest that practically all of the radioiodine is associated with the protein fraction of the milk. If these figures were applied to I¹³¹ contamination of bovine milk, it would suggest that I¹³¹ would be found in such dairy products as skim milk, cottage cheese, and whey. Butter, cream and cream products, on the other hand, would contain very little I¹³¹.

Blood Constituents and Blood Volume

There were no significant changes in any of the blood constituents except in the protein-bound iodine fraction in the three ewes remaining in the group fed 15 μc/day since August, 1951. The mean PBI value for the first five months of 1953 for this group was 2 μg/100 ml compared with control values of over 3 μg/100 ml.

Blood volume determinations were performed on 18 sheep including a thyroidectomized and an I^{131} treated hypothyroid animal. The blood volume expressed in ml/kg body weight showed a variation with weight in that the fatter animals had a lower ratio of blood volume to unit weight. However, the blood volumes of the two treated animals approximated that of controls of comparable weight. Control values of 50 to 65 ml blood/kg body weight were obtained for animals weighing from 50 to 100 kg.

Supplemental investigations under way relating to the toxicity of I^{131} include the following: (1) a study relating the μc of I^{131} /g of vegetation to the thyroid uptake of grazing sheep following a single contamination; (2) a study to determine the chronic toxicity of I^{131} to pigs on high and low planes of nutrition; and (3) a study to determine the fetal thyroid weight and I^{131} concentration at various stages of gestation.

ACKNOWLEDGMENTS

The valuable assistance of R. L. Browning, Biology Control Unit; M. E. Kerr and the Animal Farm staff; Patricia L. Hackett and personnel of the Clinical Laboratory; and N. L. Dockum and Elizabeth J. Coleman of the Microscopy group is gratefully acknowledged.

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Progress Report

TOXICITY OF I¹³¹ IN SHEEP

XI. HISTOLOGIC EFFECTS IN THYROID GLAND AT FIVE μ c/DAY LEVEL

S. Marks, N. L. Dockum, and L. K. Bustad

The histopathologic effects observed in lambs and ewes at a level of administration of 5 μ c/day are described. The usual pathologic findings consist of interfollicular fibrosis in the adult ewes and interfollicular edema in the lambs.

The lowest level of chronically administered I¹³¹ described as effective in causing damage in the thyroid gland of sheep was the 5 μ c/day level (1). The effects observed in this group were of a low order of magnitude and were initially observed after relatively long periods of feeding. For these reasons, the damage at this level of exposure may be regarded as approaching a minimal effect. This paper summarizes the findings in different age groups of sheep fed 5 μ c/day.

METHODS

The experimental material included in this report comprises four original ewes, eight yearlings or two-year-olds, and 45 lambs. The period of administration of I¹³¹ to the original ewes varied from 22 to 44 months. The lambs included in this study varied in age from birth to 27 months.

RESULTS AND DISCUSSION

Damage was not observed at 22 months but was apparent at 35 months in the original ewes. The lambs were exposed to the I¹³¹ in utero during fetal life, by way of the maternal milk until weaning at four

months of age, and subsequently by means of direct feeding in spiked feed pellets. The intake of I^{131} obviously varies during the different periods of development. No pathologic effects were observed microscopically prior to the age of eight months.

The estimated radiation dosage (2) in the animals in this study is presented in Table 1.

The histologic effects in two adult ewes consisted of the diffuse presence of minimal fibrosis between the thyroid follicles. The fibrous tissue was relatively delicate and caused a slight separation of the follicles (Figure 1). The epithelium of the follicles showed no manifest changes. The principal finding in the lamb thyroids was the presence of edema between the follicles. The edema tended to exhibit a focal distribution, groups of the follicles showing a variable degree of separation. In areas of mild involvement, the follicles were rounded, losing the surface contact and mutual deformation observed in the normal pattern (Figure 2). In the presence of more pronounced damage, the appearance was that of follicles suspended in a fluid medium (Figure 3). At an age of 27 months, a lamb thyroid showed the presence of the interfollicular fibrous tissue observed in the adult ewes. An exception to the usual appearance was observed in the thyroid of a 15-month-old lamb in the form of a focus of chronic inflammation surrounding a few damaged follicles (Figure 4). The remainder of the gland was unaffected.

Further studies are being conducted at levels of administration of I^{131} of 0.15, 0.5, and 1.5 $\mu\text{c}/\text{day}$ to define more precisely the level of minimal damage and to establish the nature of any pathologic findings which might occur at lower levels. In addition, more animals in the 5 $\mu\text{c}/\text{day}$ group will be sacrificed.

TABLE 1

Estimated Radiation Dosage and Its Effect in Varying Age Groups

Animal Number	Age I^{131} Feeding Begun (months)	No. Months Exposed to $5 \mu c I^{131}$	Total Dose to Thyroid in rad*	Histologic Damage to Thyroid
11-21	27	22	15,000	None
11-16	27	35	20,000	Yes
11-13	27	43	25,000	Yes
11-20	27	44	20,000	None
31-71	in utero	27	9,000	Yes
21-20	in utero	16	19,000	Yes
41-182	in utero	16	13,000	Yes
101-179	in utero	16	10,000	Yes
101-137	in utero	15	12,000	Yes
41-140	in utero	15	10,000	Yes
101-191	in utero	14	12,000	Yes
111-251	in utero	8	6,000	Yes
111-280	in utero	8	5,000	None

* Radiation dose does not include fetal exposure since the data for the estimation of in utero exposure are incomplete at this time.

No lambs below the age of eight months are included in the Table.

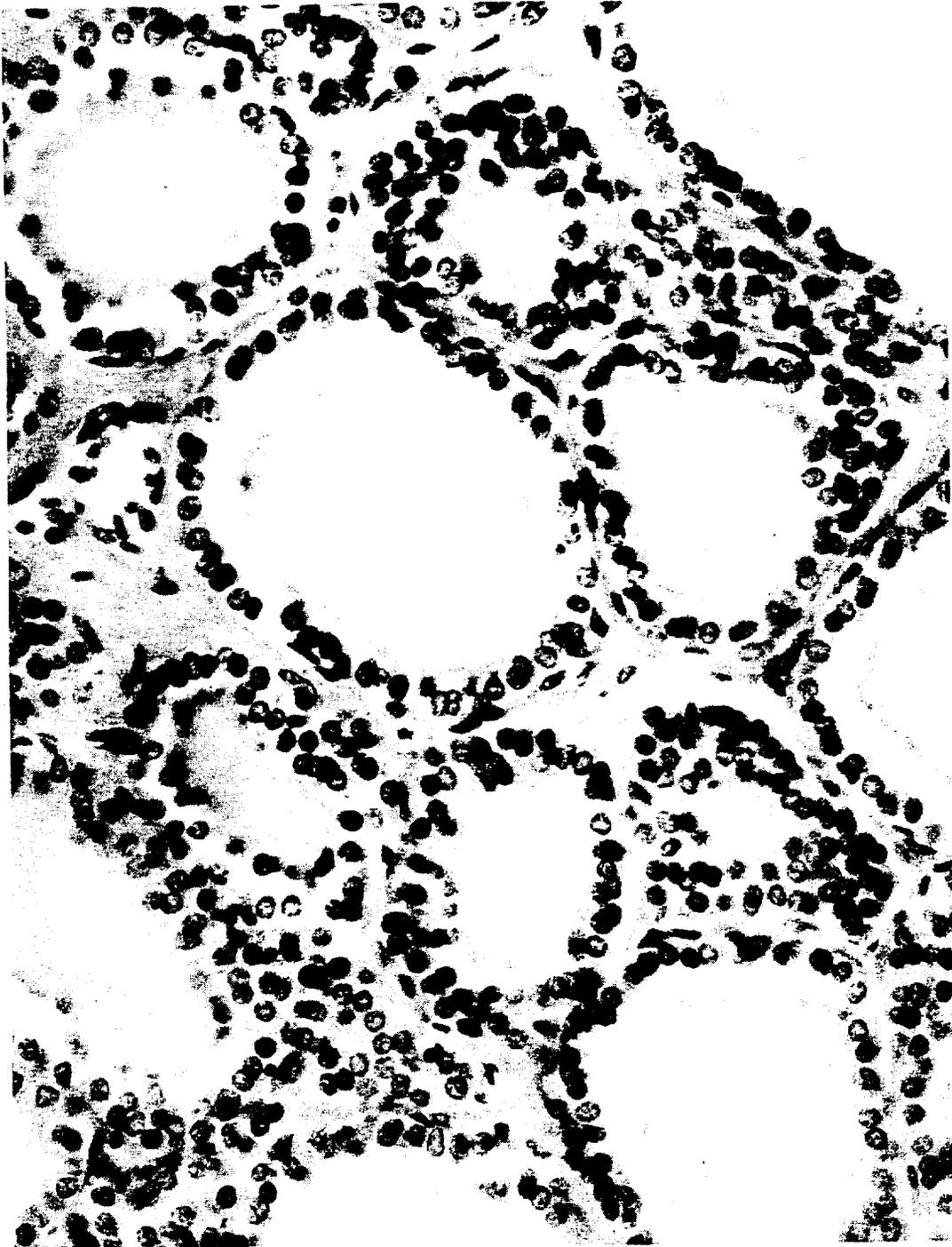


FIGURE 1. MINIMAL FIBROSIS BETWEEN FOLLICLES IN THYROID GLAND OF ADULT EWE.

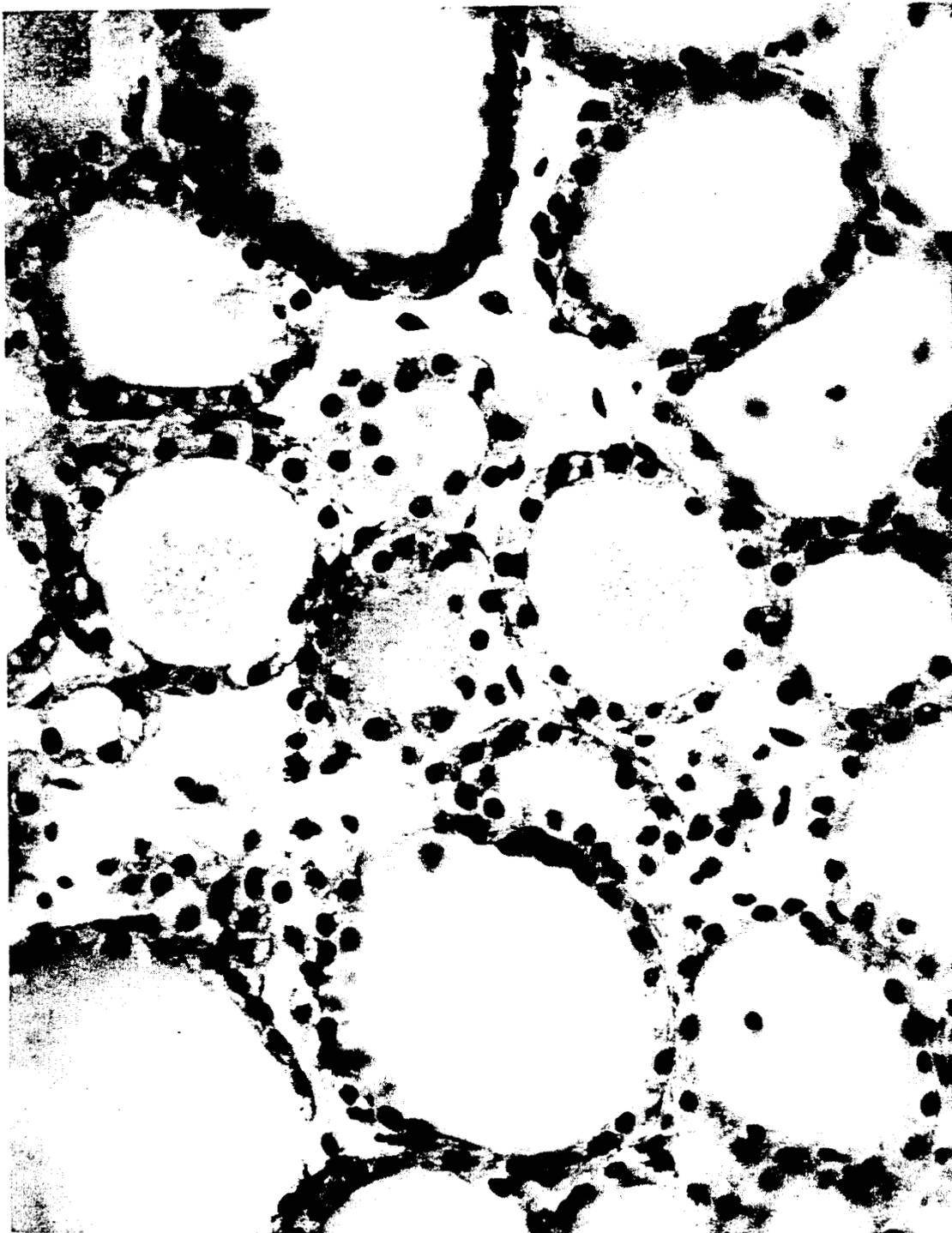


FIGURE 2. SLIGHT EDEMA BETWEEN FOLLICLES IN THYROID GLAND OF 14-MONTH-OLD EWE.

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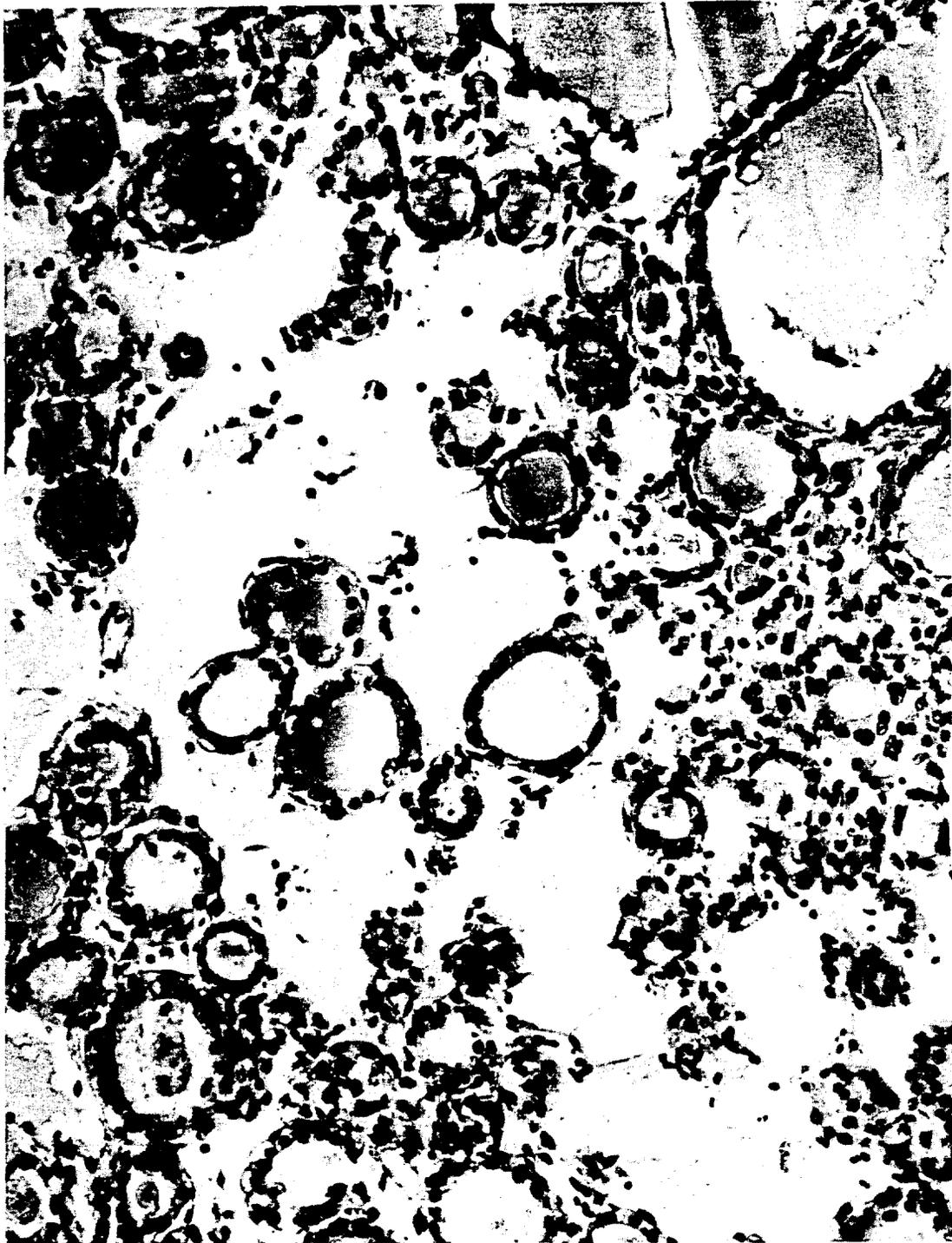


FIGURE 3. MODERATELY SEVERE EDEMA IN STROMA OF THYROID GLAND OF 14-MONTH-OLD EWE.

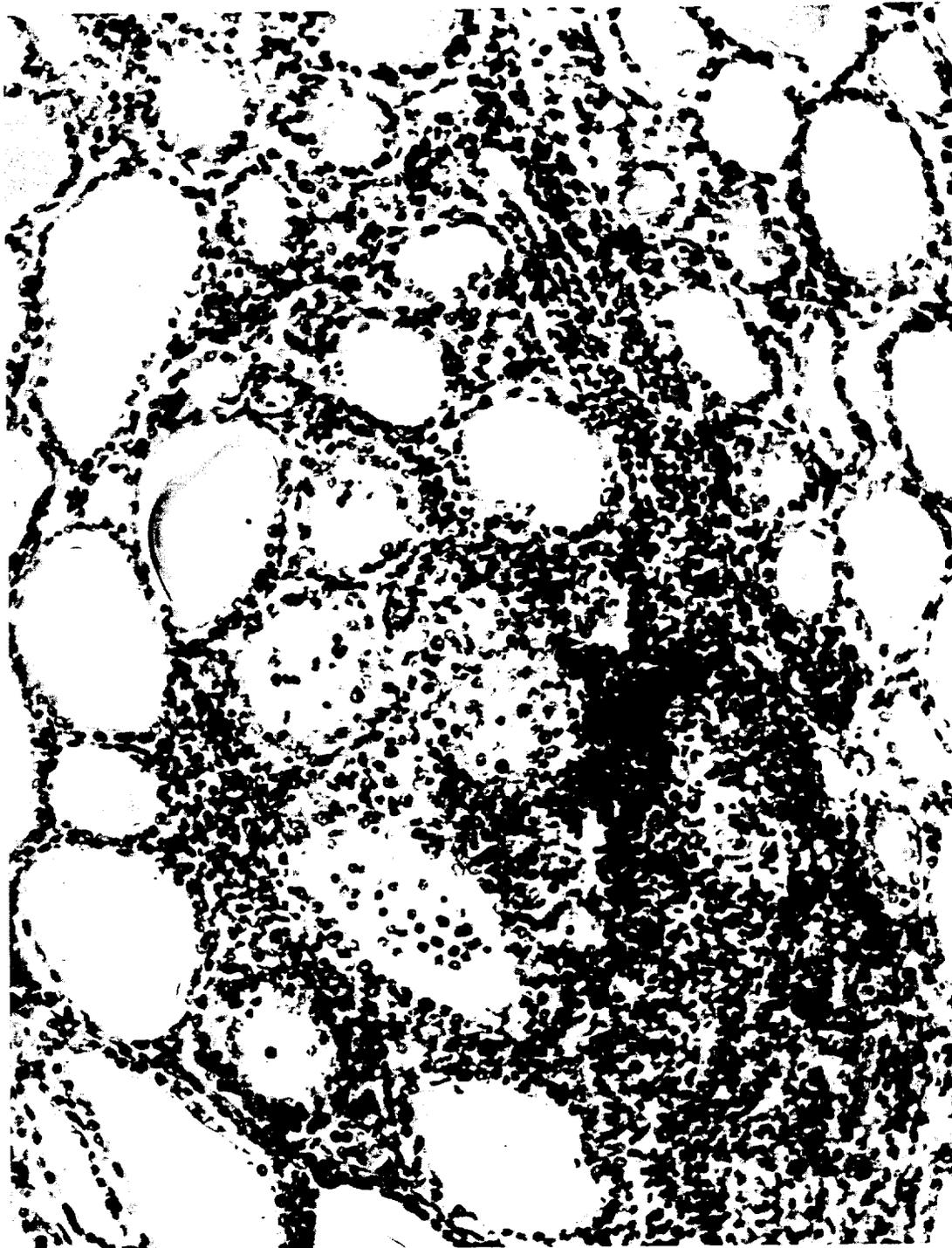
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FIGURE 4. DENSE LYMPHOCYTIC INFILTRATION IN LOCALIZED AREA OF DAMAGE IN THYROID GLAND OF 15-MONTH-OLD EWE.

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Progress Report

TOXICITY OF I¹³¹ IN SHEEPXII. EFFECT ON GONADAL FUNCTION OF THE RAM

D. E. Warner, S. Marks, and L. K. Bustad

No apparent effects on the gonads of rams exposed to thyroid ablative amounts of I¹³¹ over a period of two years were observed.

A small-scale study as a supplement to an extended chronic study on the toxicity of I¹³¹ (1) was initiated to determine if the gonads were affected in rams chronically fed thyroid-damaging amounts of I¹³¹. This is a report on the effects of various levels of daily I¹³¹ administration on the libido, spermatozoa and gonadal histology of rams.

METHODS

In 1950, nine ram lambs were divided into three groups at weaning age (four months). Nine animals were also added at weaning in 1951 (Table 1).

TABLE 1

Groups and Amounts of I¹³¹ Fed

Year	1950			1951		
Amounts of I ¹³¹ Fed Daily	Control	5 μ c	240 μ c	Control	45 μ c	135 μ c
Number/group	3	3	3	3	3	3
Exposure period (mos.)	35	8, 35 and 35	5, 8 and 11	24	11, 24 and 24	7, 20 and 21

Thyroid monitoring was performed weekly by instruments described previously (2) in order to assess damage to ram thyroids.

Collection of semen was made by means of an artificial vagina, tipped with a plastic centrifuge cone of 5 ml capacity. The ram was permitted to mount a ewe, who was either in normal or artificially induced estrus, and the penis diverted into the artificial vagina. Samples of semen were successfully obtained by this technique in all except the 240 μc group in which weakness prevented mounting of the ewe. The ejaculate obtained was immediately transferred in its plastic cone to a water bath at 37^oC. Aliquots were examined for initial motility, concentration (by direct count in a hemocytometer chamber), and the reduction of methylene blue and resazurin dyes by the semen. One technique used on all samples was the "Dead - Alive" staining test (3). Libido was assessed in all cases by the observation of behavior in the presence of ewes in estrus. Histological preparations were made on testicular tissue at necropsy.

RESULTS AND DISCUSSION

No demonstrable effects were observed in the semen or the testes of rams administered daily feedings of 5, 45, 135 and 240 μc of I¹³¹. The concentration, motility and staining quality of the spermatozoa were normal. The testes showed no fibrosis or microscopic alteration in the epithelium of the seminiferous tubules. A diminution in the libido was apparent only in the presence of the depression accompanying the severe hypothyroid state.

ACKNOWLEDGMENTS

Appreciation is expressed for the assistance of M. E. Kerr and the Animal Farm staff; and to N. L. Dockum and Elizabeth J. Coleman for histological preparations.

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Progress Report

TOXICITY OF I¹³¹ IN SHEEPXIII. THE EFFECT OF I¹²⁷ ON I¹³¹ UPTAKE IN SHEEP

L. K. Bustad and D. E. Warner

Supplying iodized salt (in levels approximating those used by many farmers) to sheep on a marginally goitrogenic diet reduced thyroid uptake of a single tracer dose of I¹³¹ by over 30 per cent. Inorganic iodide administered daily at levels of 2.5 mg to sheep maintained on 5 and 0.15 μ c of I¹³¹ daily reduced thyroid uptake to levels less than 50 per cent of the controls.

This paper reports the results of two supplemental studies designed to evaluate the degree of depression in thyroid uptake of I¹³¹ that occurs in the presence of near normal intakes of I¹²⁷ compared with the uptake of I¹³¹ in the presence of marginally goitrogenic levels of stable iodine.

In the main experiment, designed to define the toxicity of radioiodine, the sheep are maintained on a low iodine intake (1). This is done in order to simulate range conditions of the plant environs which are considered a marginally goitrogenic area (2) and to insure maximum thyroid uptake of I¹³¹. Although it is known that I¹²⁷ reduces the amount of I¹³¹ subsequently taken up by the thyroid gland (3), quantitative data relating specific dosages to the thyroid uptake are lacking.

Knowledge of this type is essential to the application of data obtained in experiments conducted under marginally goitrogenic conditions to the analysis of observations on sheep fed a diet containing substantial iodine. This information could also serve as a basis for the estimation of the supplemental feedings of iodine required to minimize thyroid damage in the event of a high level of contamination.

METHODS

In one study 19 yearling Suffolk sheep were given a 30 μc tracer dose of I^{131} . Nine animals of this group were then placed on a feeding regimen which included one-half pound of grain mixture containing one-fourth ounces of iodized salt (including 0.01 per cent KI) and were allowed free access to additional iodized salt. This method of administering iodized salt is commonly used by many farmers in this area. The nine animals given iodized salt were taken from the off-project control flock. A second 30 μc tracer dose of I^{131} was administered when the thyroid glands exhibited one μc or less of I^{131} remaining from the first dose as revealed by external monitoring (4). Ten of the animals served as controls and did not receive iodized salt nor had they received any during their lifetime as members of the on-project control group.

In the second supplemental experiment involving 15 yearling Suffolk ewes, one-half of each of two groups receiving 5 and 0.15 μc of I^{131} per day were given 2.5 mg I^{127} orally per day as KI. Thyroid monitoring was performed one or two times daily during the first week and two to three times per week thereafter.

RESULTS AND DISCUSSION

The results of the second 30 μc tracer dose are shown in Figure 1. It will be noted that the mean level of μc in the thyroid at 40 hours in the group, which was placed on iodized salt 18 days previously, is only about 55 per cent of that of the control group. Following the first tracer dose, thyroidal uptake of I^{131} in the subsequently treated group was about 82 per cent of the level reached by the control group.

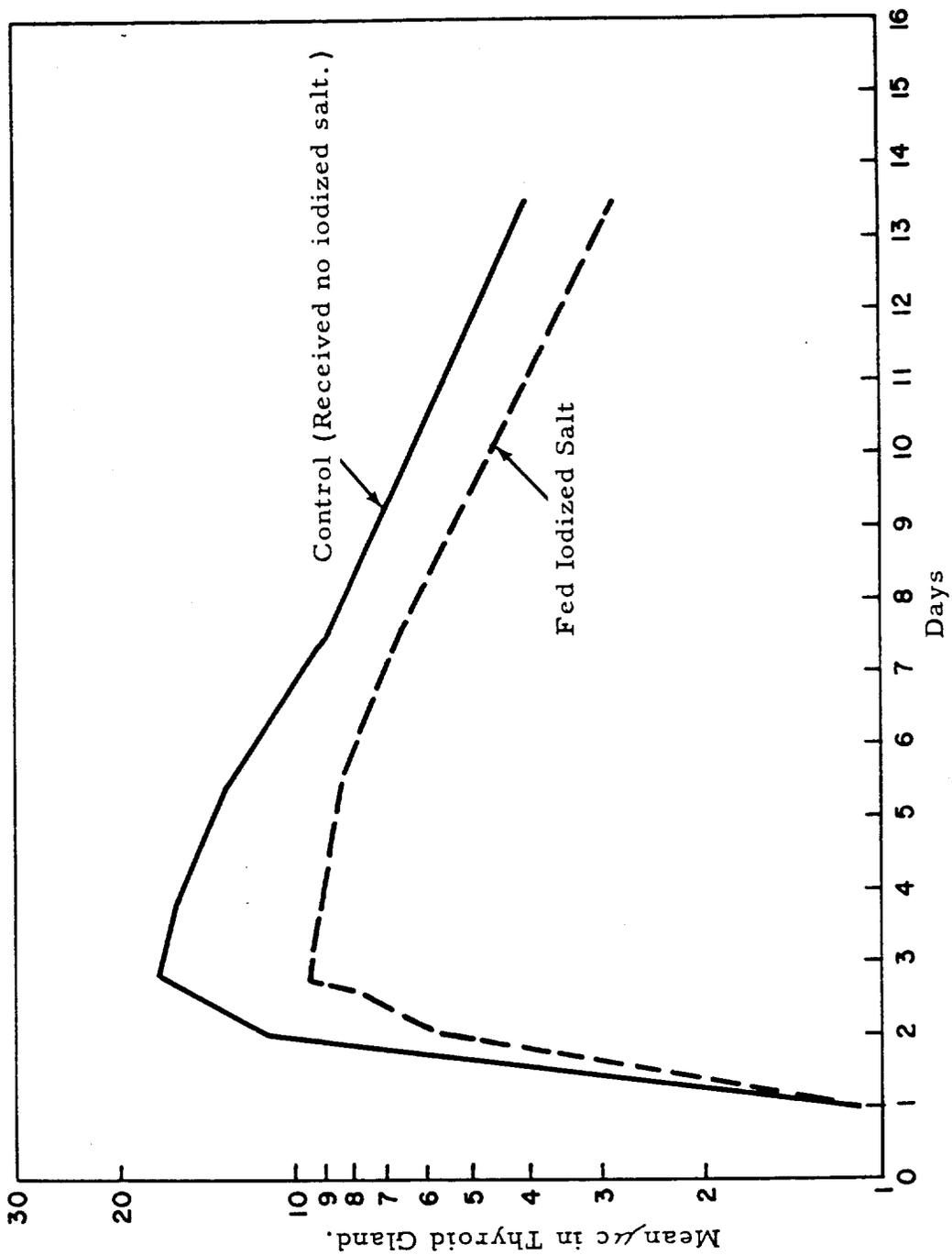


FIGURE 1
EFFECT OF IODIZED SALT ON THYROID UPTAKE IN SHEEP FED
30 μc TRACER DOSES OF I¹³¹.

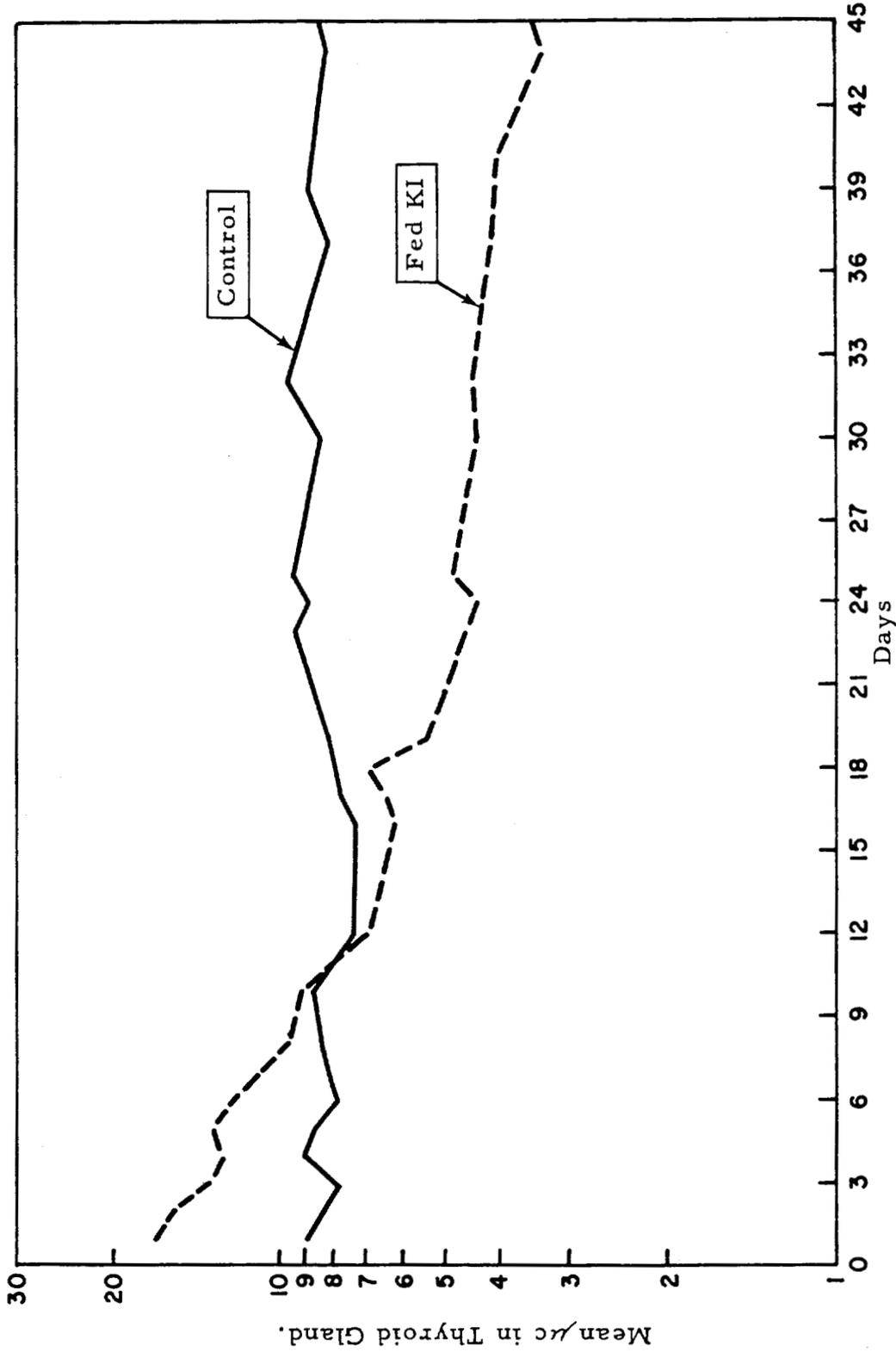


FIGURE 2
EFFECT OF DAILY SUPPLEMENT OF KI ON THYROIDAL IODINE IN SHEEP
FED 5μc OF I¹³¹/DAY.

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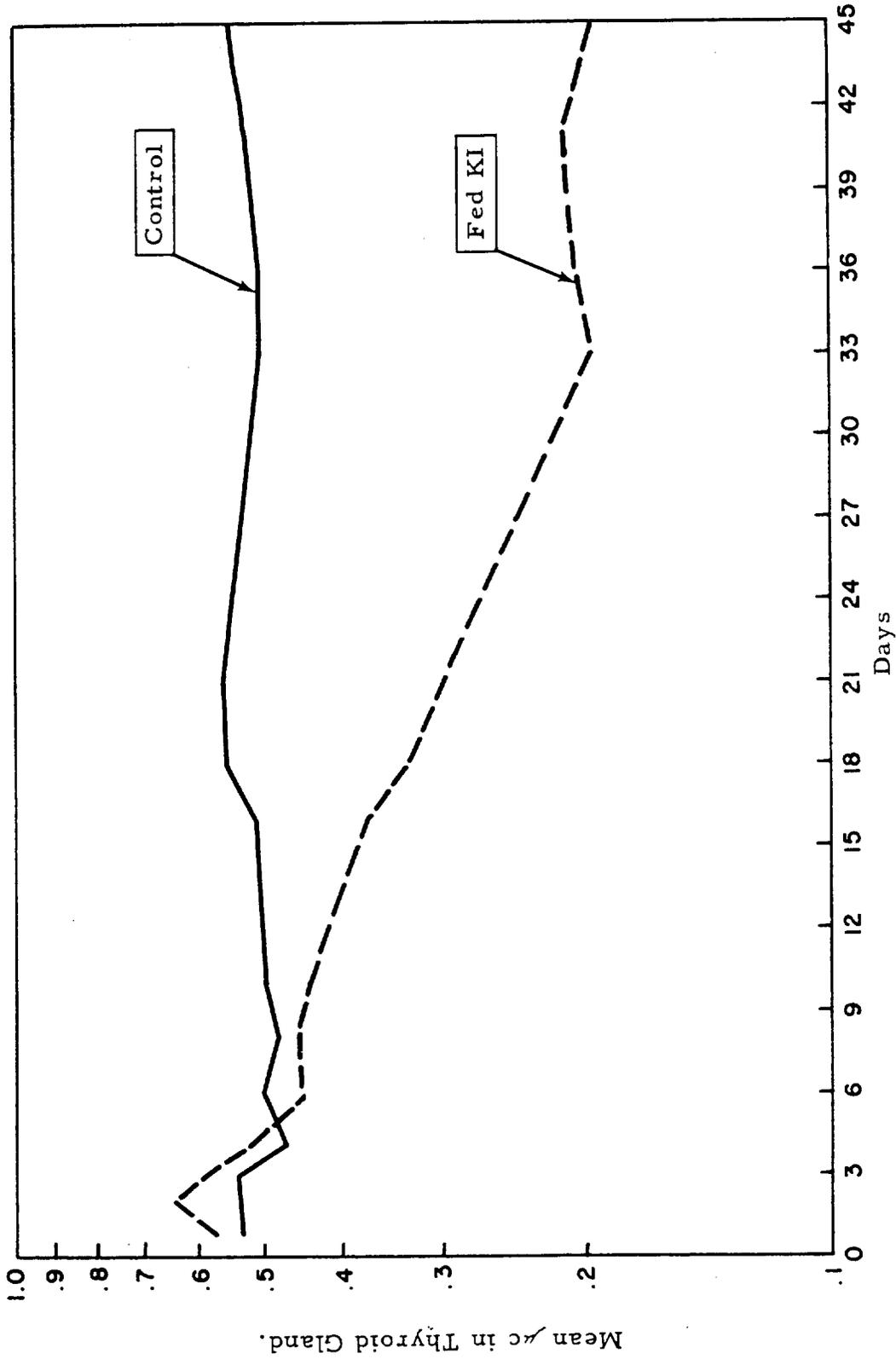


FIGURE 3
EFFECT OF DAILY SUPPLEMENT OF KI ON THYROIDAL IODINE IN SHEEP
FED 0.15 μc OF I^{131} /DAY.

The results of the second supplemental study are found in Figures 2 and 3. Within a 30-day period the thyroid content of I^{131} as determined by external monitoring was reduced to less than one-half that of the controls.

These data suggest that the uptake of I^{131} by the thyroid gland of sheep on farms where iodized salt is fed may be about one-half that observed under goitrogenic conditions maintained in our main experiment.

ACKNOWLEDGMENTS

Grateful acknowledgment is made for compilation of data by Margaret E. Kerr and for care, feeding and monitoring of animals by Malcolm E. Kerr and staff of the Experimental Animal Farm.

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Progress Report

BIOLOGICAL MONITORING

W. C. Hanson and R. L. Browning

The second year of investigation of the ratio (thyroid activity density)/(I¹³¹ emission rate) in rabbits collected on the HAPO project was completed. A pattern of variation comparable to that observed in previous months (Figure 1) was resumed following a period of great fluctuation due to unseasonal weather. The pronounced increase in the ratio which occurred in June was assumed to be due, in part, to a local rain-out of airborne contamination that originated off-site. Unseasonal mild weather which reduced wind dilution of stack gases is believed responsible for the high ratio during December.

Detectable levels of fission product contamination were noted in feces samples of all specimens collected during the latter half of the year.

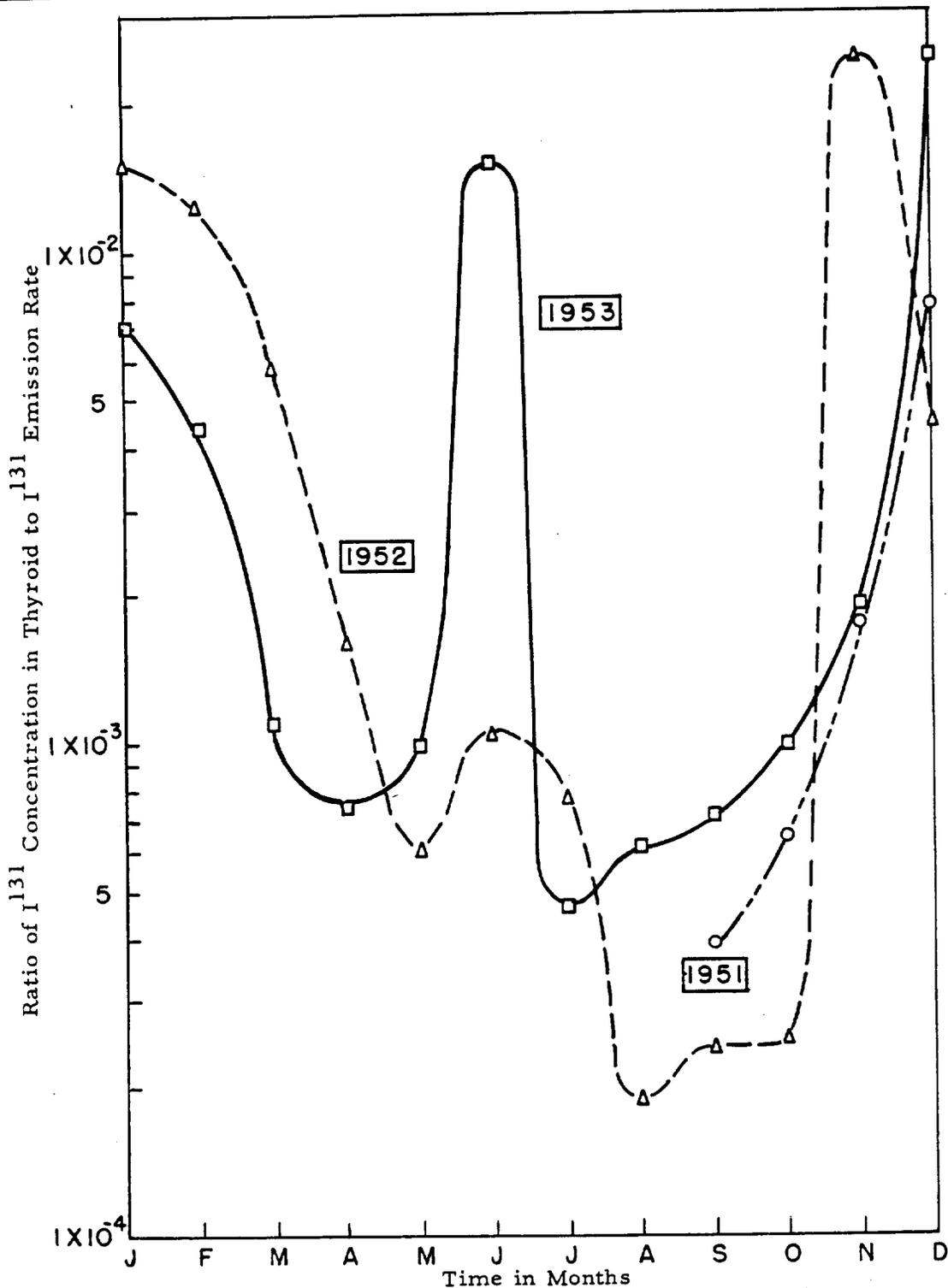


FIGURE 1
RELATION OF THYROID ACTIVITY DENSITY TO I^{131} EMISSION RATE
($\mu\text{c } I^{131} / \text{g THYROID TISSUE}$) / (CURIE I^{131} EMITTED).

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Davis, J. J., "The effects of pile effluent water on Columbia River organisms," March 3, 1953, Washington State College Seminar (Enzymology Group), Pullman, Washington.

Hungate, F. P., "The use of radioisotopes in biochemical genetics," March 5, 1953, Washington State College Seminar (Genetics Group), Pullman, Washington.

Thompson, R. C., "Studies of metabolic turnover with tritium as a tracer," May 27, 1953, American Chemical Society, Richland, Washington.

Kornberg, H. A., "Biological effects of radiation," July 7, 1953, Annual Meeting of Science Teachers, Oregon State College, Corvallis, Oregon.

Kornberg, H. A., "Radioactive tracers," July 8, 1953, Annual Meeting of Science Teachers, Oregon State College, Corvallis, Oregon.

Palmiter, C. C., "Effects of pile effluent waters upon Columbia River organisms," August 6, 1953, Seminar at Montana State University Field Station - Flathead Lake, Montana.

Bustad, L. K., "Toxicity of radioiodine," August 1953, XV International Veterinary Congress, Stockholm, Sweden.

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Porter, J. W., "Further studies on the action of growth-inhibiting levels of tritium oxide on Chlorella pyrenoidosa," September 8, 1953, American Society of Plant Physiologists, Madison, Wisconsin.

Whittaker, R. H., "The distributional meaning of plant and animal associations," September 8, 1953, Ecological Society of America, Madison, Wisconsin.

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Kornberg, H. A., "Some implications of contemporary biology," December 17, 1953, 1953 Annual Conference - "Looking Ahead Into Science," Oregon State College, Corvallis, Oregon.