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BIOLOGY RESEARCH - ANNUAL REPORT

1954

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By

The Staff of the
Biology Section

H. A. Kornberg, Manager

J K Soldat 11100

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1954 ANNUAL REPORT - BIOLOGY SECTION

INTRODUCTION

This volume is the fourth in a series that annually summarize the section's research activities. The immediately preceding volume is HW-30437, for the calendar year 1953.

Topically, the thirty-one papers that comprise this report fall into five categories as follows:

1. Effects of reactor effluent on aquatic and plant life.
2. Plant and animal absorption and metabolism of several fission products, plutonium, and tritium.
3. Effects of I^{131} and external radiations administered to sheep.
4. Toxicity of radioactive particles.
5. New or improved techniques of possible application to radiobiology.

Appreciation is expressed to Dr. R. F. Foster who assisted in editing this report. Authors wish to acknowledge the analytical services performed for them by A. C. Case and personnel of the Radiochemistry Laboratory.

H. A. Kornberg

1954 RADIOBIOLOGICAL SURVEY OF THE COLUMBIA RIVER

J. J. Davis, D. G. Watson and C. C. Palmiter

The distribution of radioactivity among river organisms was comparable to that found in 1953. In the newly formed McNary Dam impoundment, activity densities of plankton and bottom organisms were lower along the Oregon side owing to dilution from the Snake River. The number of salmon nests between Richland and Priest Rapids was comparable to those observed during 1953, but was appreciably lower than the number for the parent year of 1950; the reduction was commensurate with small runs of salmon to other sections of the Columbia.

Sampling of Columbia River organisms for radioanalyses and ecological studies was continued during 1954 to evaluate radiation hazards and to determine effects of reactor effluents upon the river biota. Sampling and process methods have been previously described (1, 2). Since the differences in activity densities among most species of Columbia River organisms, and the seasonal and geographical patterns have already been determined (1, 2, 3), continued monitoring was adequately accomplished by a reduced sampling schedule. This schedule included the collection of plankton and representative species of small fish and invertebrates twice a month from two stations near Hanford, monthly from Richland and the vicinity of McNary Dam, quarterly from Priest Rapids, and the quarterly gill-netting of large fish at Hanford. Special attention was given to studies of the newly formed McNary Dam reservoir to determine the effects of the impoundment upon the activity densities in aquatic organisms, and to evaluate the ecological effects.

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RESULTS AND DISCUSSION

The relative levels of radioactivity attained by the various species of river organisms and their seasonal patterns did not differ significantly from those of previous years (1, 3). The geographical distribution of activity from Hanford to the mouth of the river, as determined by routine sampling and a special survey of the lower river, was approximately the same as reported for last year (2).

Decay curves and radiochemical analyses were made of representative organisms collected at stations from Hanford down to the mouth of the river. While only trace amounts of radioisotopes with less than 14 day half-lives were found in fish and bottom invertebrates collected at distances greater than 20 miles below the reactors, detectable amounts of short-lived activity persisted in plankton sampled seven miles below McNary Dam. This was expected because of the position of plankton in the food chain and because it travels freely with the water currents. In the vicinity of Hanford, Cu^{64} was responsible for 1 to 7.5 per cent of the total activity in small fish. It accounted for 8 to 17 per cent of the total in snails, caddis fly larvae and filamentous algae and about 25 to 35 per cent in plankton. Na^{24} made up 3 to 14 per cent of the total in all types of samples analyzed.

The distribution of radioactivity in the McNary Dam inpoundment was determined for plankton and bottom organisms. Plankton samples were taken on cross-sectional traverses at Richland (mile 339*), at mile 324.5, Cold Springs (mile 300) and just above McNary Dam (mile 292.5).

---

* Mileage figures are distances from the mouth of the Columbia based upon the USPHA report "Report of Pollution Control Subcommittee Work-Group on Columbia River and Tributaries Mileage Index," October 20, 1952.

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Bottom organisms were dredged on a cross-sectional traverse at Cold Springs. No tributaries enter the Columbia between the reactors and Richland; and radioactivity was quite uniformly distributed across the upper end of the impoundment in the vicinity of Richland. The Yakima River, with a mean flow of 3,900 cfs, enters the Columbia immediately below Richland, but does not significantly influence the geographical distribution of activity density in plankton at Pasco 6 miles downstream. A short distance below Pasco the Columbia receives an average of 50,600 cfs from the Snake River which reduces activity densities along the Oregon side of the impoundment. This was particularly evident in bloodworms dredged at Cold Springs during the latter part of the year as shown in Figure 1.

Between November, 1953, and October, 1954, 41 per cent of the whitefish sampled from the Priest Rapids sportfishing area, upriver from the reactors, had activity densities above background levels. The average activity density of fish sampled from this location was slightly higher than for 1953. Whitefish sampled from near Ringold, immediately downstream from the restricted area of the reservation, generally had slightly higher activity densities than those from Priest Rapids.

A total of 157 chinook salmon nests was observed between Richland and Priest Rapids. This was comparable to the 149 nests reported for 1953, but appreciably lower than the 290 observed during the assumed parent year of 1950. The reduced number of local nests is commensurable with the subnormal fall run of salmon in the Columbia River as a whole.

There was no evidence that aquatic life of the Columbia River has been adversely affected by effluent from the Hanford reactors.

ACKNOWLEDGMENTS

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

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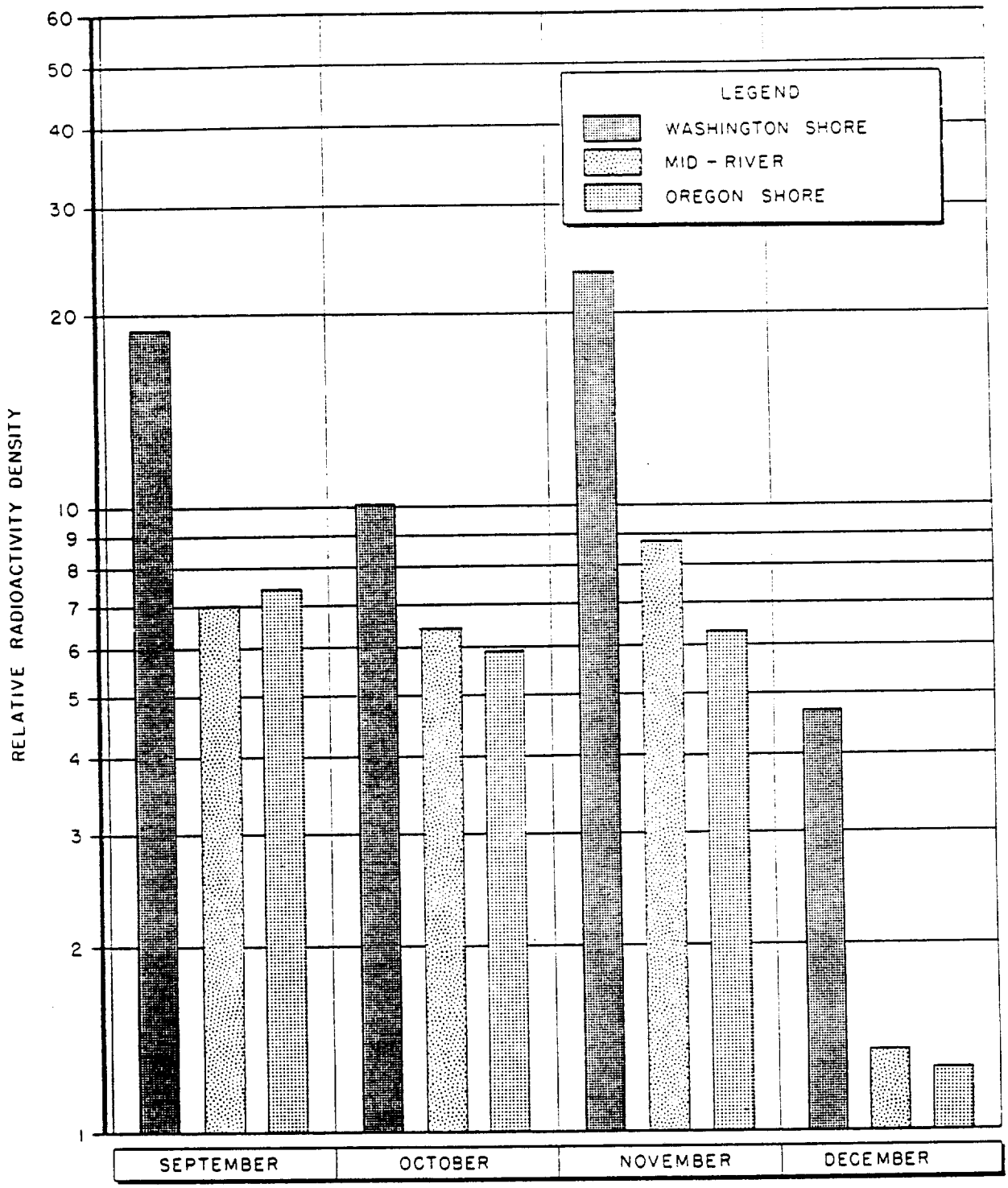

FIGURE I.
RADIOACTIVITY OF BLOODWORMS IN
MC NARY RESERVOIR - 1954

AEC-GE RICHLAND WASH

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REACTOR EFFLUENT MONITORING WITH YOUNG CHINOOK SALMON - 1954

P. A. Olson and R. F. Foster

Chinook salmon eggs and young were subjected to a series of dilutions of dechlorinated reactor influent and reactor area effluent for a period of seven months. A slight increase in mortality occurred among the fry held in 5 per cent influent and growth was slightly retarded in the 3 per cent influent. Although a detectable mortality resulted among eggs incubated in one per cent effluent this probably resulted from atypically high temperature. More severe loss of eggs and young fish in higher effluent concentrations is attributed to unfavorably high temperature and dichromate content. The amount of radiation received was low and presumably did not contribute to the effect. Extrapolation to conditions existing in the river indicates no adverse effect on the young salmon.

Earlier reports have described the objectives of this series of studies where young salmon are subjected to dilutions of the reactor effluent water (1, 2, 3). Last year's report (4) covered a period ending in October, 1953; the work reported here describes results of the effluent monitoring with chinook salmon from November 1953, to May 1954. The 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 monitor biologically the quality of the effluent being discharged by a typical reactor installation.

MATERIALS AND METHODS

Approximately 49,000 chinook salmon eggs, obtained from the Washington State Department of Fisheries hatchery at Soos Creek (Puget Sound drainage), were randomly distributed among sixteen troughs with

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the following conditions: Columbia River water (3 troughs used as controls); dechlorinated reactor influent * at 3%, 5%, 10%, 25%, and 50%; and reactor area effluent ** at 1%, 2%, 3%, 4%, 5% and 10%. 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 measured at the end of the test. As the fish increased in size, the numbers held in each trough were reduced (on a random selection basis) to prevent overcrowding. The experiment was terminated when the young fish were of sufficient size and age for migration to the ocean.

RESULTS AND DISCUSSION

Mortalities suffered by the young salmon in the various experimental conditions are shown in Figure 1. No significant mortalities occurred among any of the egg lots incubated in dilutions of the influent water. During the fry stage, however, significant mortalities did occur in the 5 per cent and stronger mixtures. After the fish had developed to the fingerling stage, abnormally high mortalities persisted only in the 10 per cent and stronger mixtures. The growth rate appeared to be a more sensitive index of toxicity. Figure 2 shows the growth pattern for the control fish and the size reached by the fingerlings in the experimental lots. The fish grew more slowly in all cases where influent was added--even at 3 per cent strength. The adverse effects observed are largely attributed to dichromate which is added to the influent water for corrosion control. The results reported

---

* Reactor influent is the purified riverwater supplied to the reactor as a coolant. In this study residual chlorine present in the influent was removed by filtration through charcoal and the water temperature was adjusted to match that of the river.

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

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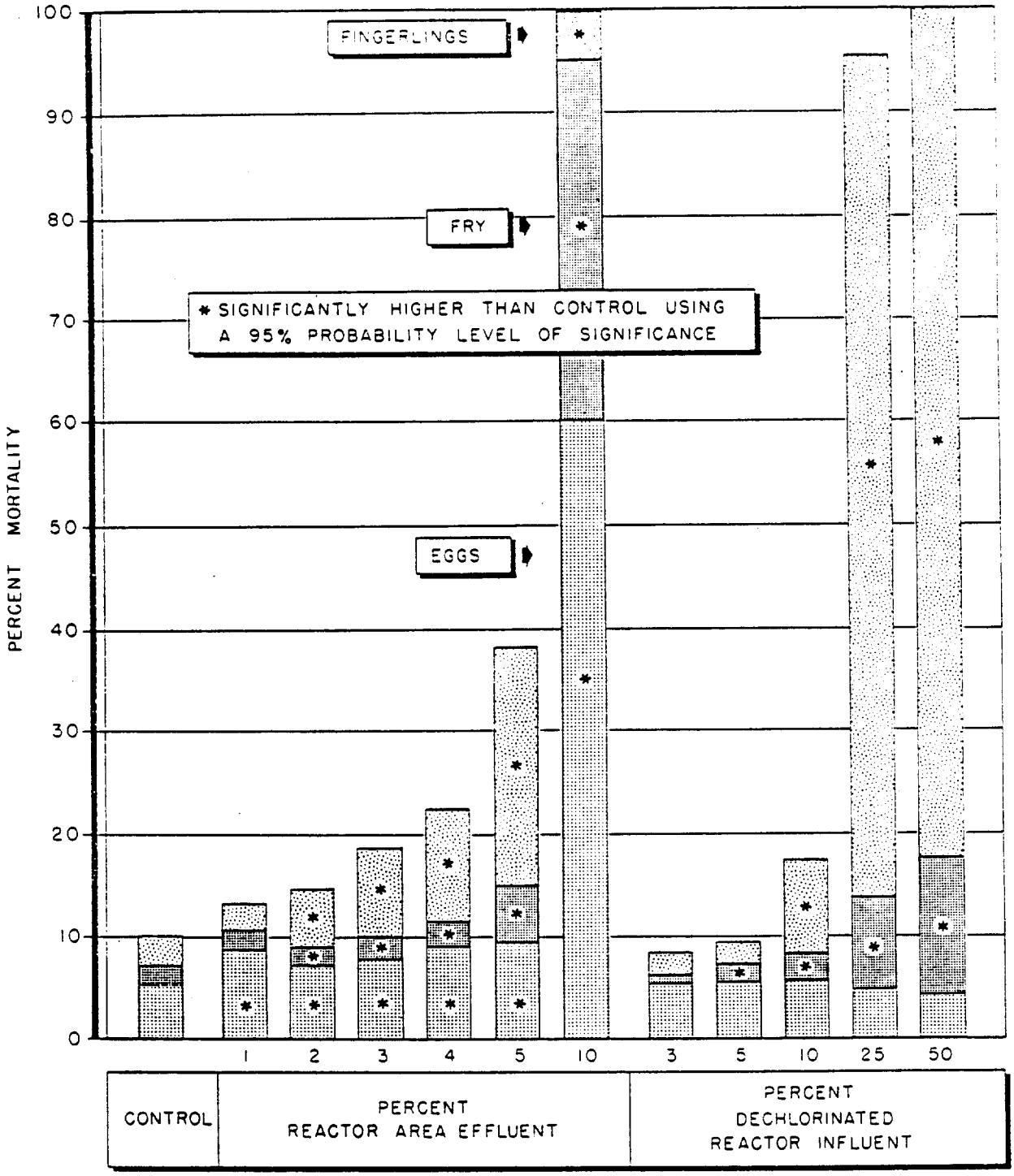

FIGURE 1
CUMULATIVE MORTALITIES OF CHINOOK SALMON IN VARIOUS CONCENTRATIONS OF PROCESS WATERS

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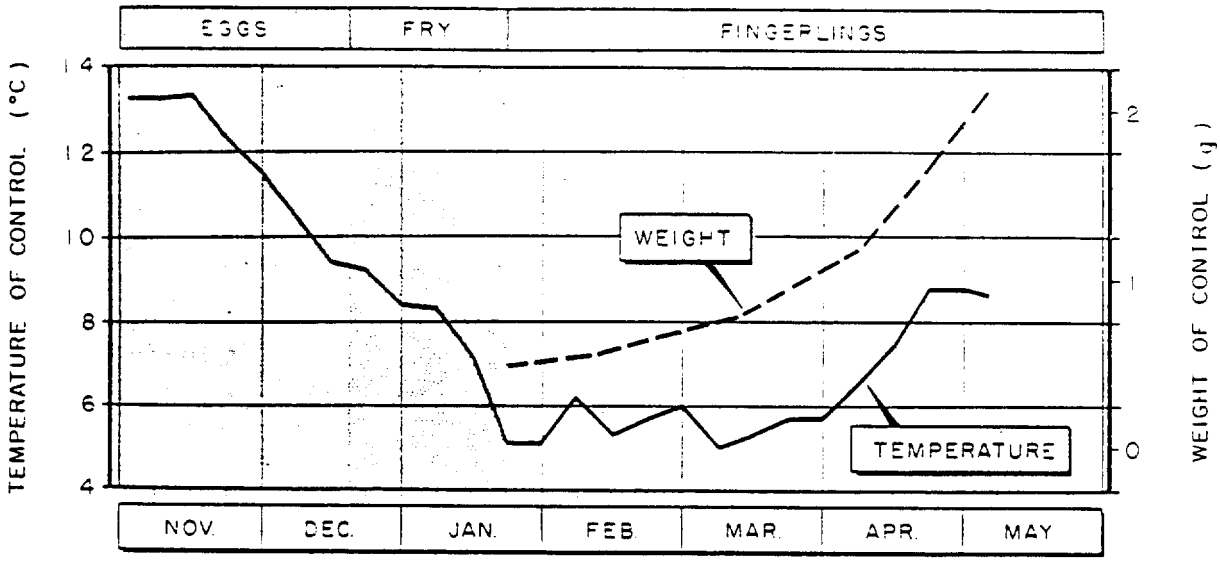

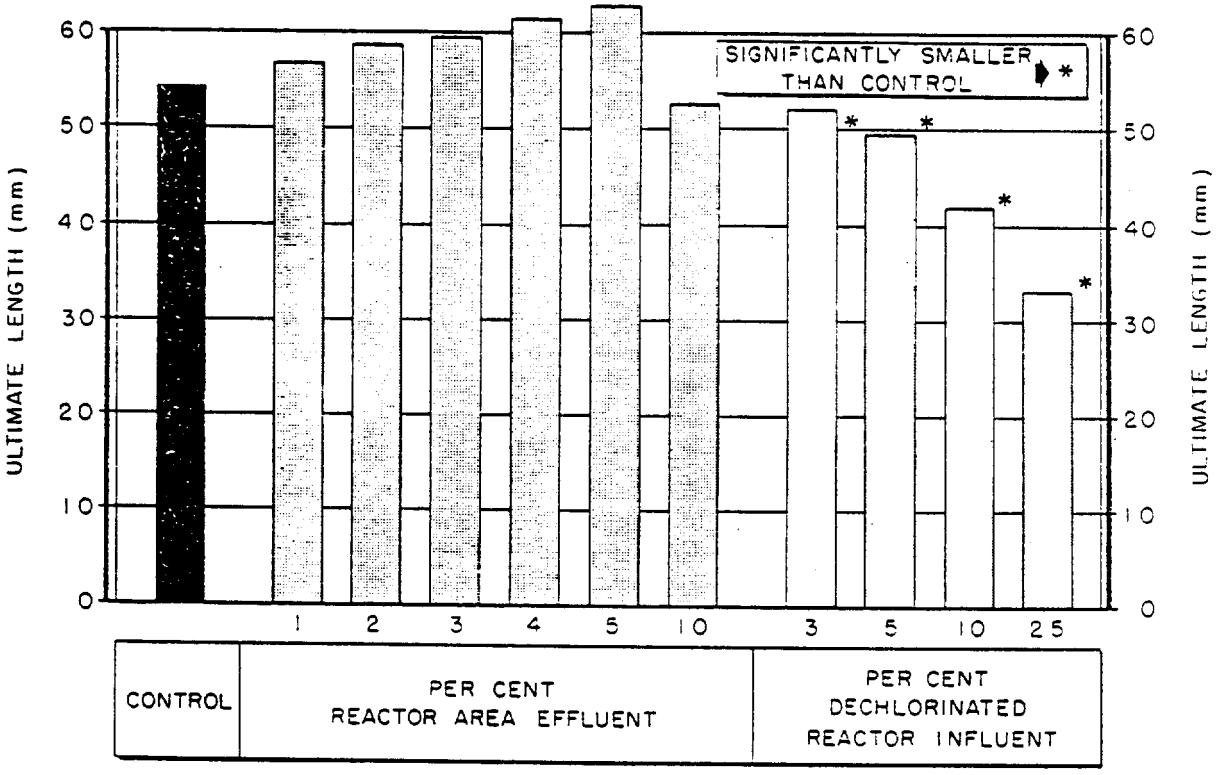

FIGURE 2
GROWTH OF CHINOOK SALMON IN VARIOUS
CONCENTRATIONS OF PROCESS WATERS

AEC-GE RICHLAND WASH

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here are appreciably different than those observed last year (4) when dichromate was not present.

In mixtures containing the effluent, some increase in mortality among the eggs was detectable at all levels including 1 per cent (Figure 1). Subsequent to hatching a significant mortality rate continued among the fry and fingerlings at the 2 per cent level and above. Virtually none of the fish survived in 10 per cent effluent. Growth rate was not a good index of toxicity in this case as indicated by Figure 2. The higher temperatures of the effluent mixtures encouraged growth and compensated for any retardation which might otherwise have occurred.

Radiochemical analyses of salmon held in the 5 per cent reactor area effluent lot indicated that about 80 per cent of the activity accumulating in the fish originated from Na^{24}, about 10 per cent from Cu^{64} and lesser amounts from As^{76}, rare earths, and isotopes of the iron, zinc-cobalt, calcium, and phosphorus groups. About 80 per cent of the gross activity was accounted for. Figure 3 illustrates the gross beta activity density of the salmon held in the 10 per cent effluent. Activity density of the eggs increased as the size of the developing embryo increased. There was an appreciable drop in activity density at the time of hatching, owing in part to the discarding of the egg shell and its absorbed radioactive materials. The activity density of the fish increased during the fry and early fingerling stages.

Since the amount of radiation received by the embryos was small, about 0.2 rad during the egg stage in 5% effluent, and since chemical toxicity was not otherwise manifested until the fry stage, the mortality which occurred among eggs incubating in mixtures of the effluent can probably be attributed to unfavorably high temperatures. Although a slight mortality resulted among eggs held in very low concentrations of the

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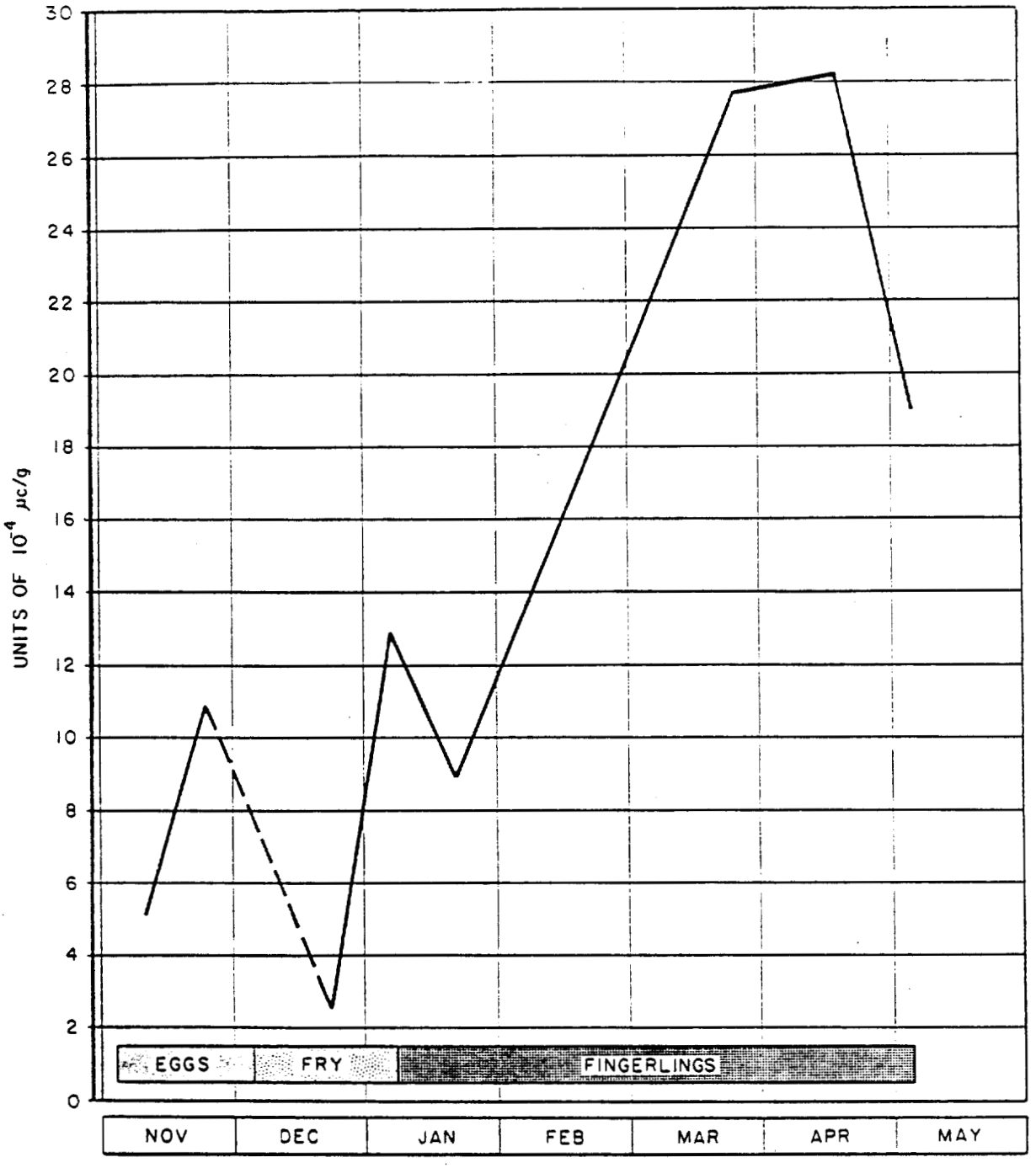

FIGURE 3
ACTIVITY DENSITY OF CHINOOK SALMON IN
10 PER CENT REACTOR AREA EFFLUENT

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effluent, this may well have resulted from atypically high temperatures in these experimental lots. This resulted from temperature gains in the river water system not conpletely compensated by heat loss of the effluent.

Inasmuch as significantly high mortalities were detected through the fingerling stage in the 2 per cent effluent, this level is considered as slightly toxic. The concentration of effluent affecting the young salmon is slightly below that observed in 1951 (2) but not inconsistent with results obtained last year (4). The toxicity is again attributed principally to elevated temperature and to dichromate rather than to radiation. Chlorine, present in the influent water, is not considered a significant toxicant to river life since it is largely dissipated by the heat and retention of the effluent.

Except for localized and well-defined areas immediately below the outfalls, reactor area effluent conditions in the Columbia River are substantially less severe than the laboratory conditions which produced adverse effects. It seems reasonable to conclude that the Hanford reactors had no effect on the young salmon of the Columbia River during this period.

ACKNOWLEDGMENTS

The assistance of Dr. L. R. Donaldson of the University of Washington in obtaining the salmon eggs is gratefully acknowledged. Statistical analyses were made by Radiation Measurement Evaluation 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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EFFECT OF REACTOR AREA EFFLUENT WATER ON CHINOOK SALMON FINGERLINGS

P. A. Olson and R. F. Foster

Fingerling-size chinook salmon from Columbia River stock tolerated 10 per cent strength reactor area effluent for three and a half months before mortality rate was affected. No adverse effect was observed at the 5 per cent level even after five months--conditions far more severe than such fingerlings would encounter in the Columbia River. Chinook fingerlings from Puget Sound stock were somewhat more sensitive to the effluent and to elevated temperature than those of Columbia River origin. Growth of the Puget Sound fish was slightly retarded in 1 per cent effluent.

Considerable numbers of chinook salmon spawn in the Columbia River and its tributaries above the Hanford Works during the fall months. Their progeny usually migrate to the ocean the following spring. These migrating fingerlings pass through the section of the Columbia River which receives effluent from reactors.

This study was designed, 1) to determine the dilution level of reactor area effluent which can be safely tolerated by fingerling chinook salmon, 2) to compare the sensitivity of the local Columbia River fingerlings with that of fingerlings from Puget Sound (Green River) which are more easily available for test animals, and 3) to perform continuous biological monitoring of the quality of the effluent discharged by the plant.

MATERIALS AND METHODS

The Columbia River fingerlings were the progeny of a single adult pair captured immediately above the reactor areas. The Puget Sound fingerlings were the progeny of several adults spawned at the Washington State

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Department of Fisheries hatchery at Soos Creek near Auburn, Washington. This test was started in May 1954, when the fish averaged 0.5 g and had just started to feed. The fingerlings were randomly distributed with 140 fish to each of 10 troughs with the following duplicated conditions: Columbia River water (control) and reactor area effluent* at 1%, 2%, 5%, and 10%.

The fish were given standard fish-cultural care. Samples from each trough were weighed at two-week intervals and the lengths of 50 fish from each lot were taken at the end of the test. The experiment was terminated in October 1954.

RESULTS AND DISCUSSION

Mortalities which occurred during the experiment are shown in Figure 1. Significant mortalities for both stocks occurred only in the 10 per cent reactor area mixtures. The Puget Sound strain showed a higher degree of sensitivity to the effluent, manifested in a greater mortality rate at the 10 per cent level.

Since the effluent may produce effects from both temperature and chemicals, data on temperature effect alone were also obtained. Twenty-five fingerlings from each of the strains were subjected to water heated to 20° C. Initially this was a temperature increment of about 7° C over the control. Mortality results were:

Local Strain	Puget Sound Strain
Control - 0%	Control - 12%
20° C - 8%	20° C - 28%

The results suggest that the local strain was more temperature tolerant than the Puget Sound strain, but the small numbers of fish used preclude precise evaluation.

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

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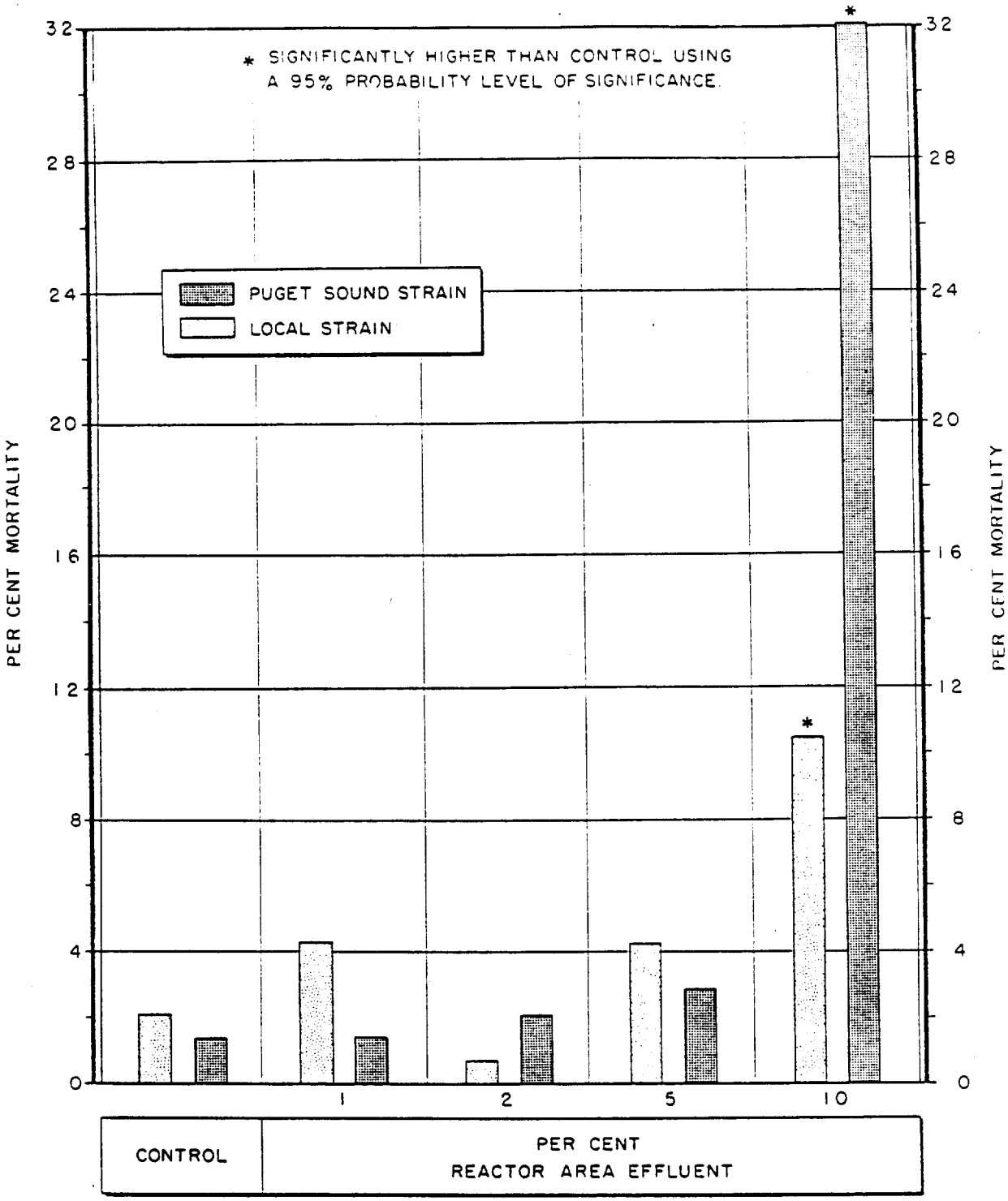

FIGURE I
MORTALITIES OF CHINOOK SALMON FINGERLINGS IN VARIOUS CONCENTRATIONS OF REACTOR AREA EFFLUENT

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Figure 2 illustrates the growth pattern of the control fish and the ultimate size attained in the various conditions. Local fish grown in the 10 per cent mixture were significantly shorter than the controls. The Puget Sound fingerlings were more sensitive to the effluent and in all effluent dilutions the fish were significantly shorter than the controls.

Field observations (1) indicate that two months is a maximum period that migrating salmon remain in the section of the river encompassed by the Hanford reservation. In this experiment the greatest mortality occurred well after the first two months.

The local strain held in the 10 per cent effluent lot showed no increased mortality even at three and one-half months. Except for areas immediately adjacent to the outfalls the concentrations of reactor effluent in the Columbia River ranged far below even the 5 per cent level. Thus all results indicate that fingerling salmon of the Columbia River should pass through this section of the Columbia en route to the ocean without harm from reactor effluent.

ACKNOWLEDGMENTS

Thanks are extended to A. C. Schroeder and the Biological Attendants who assisted with the care of the animals, operation of equipment and recording of data. Statistical analyses were made by the Control Unit of the Biophysics Section.

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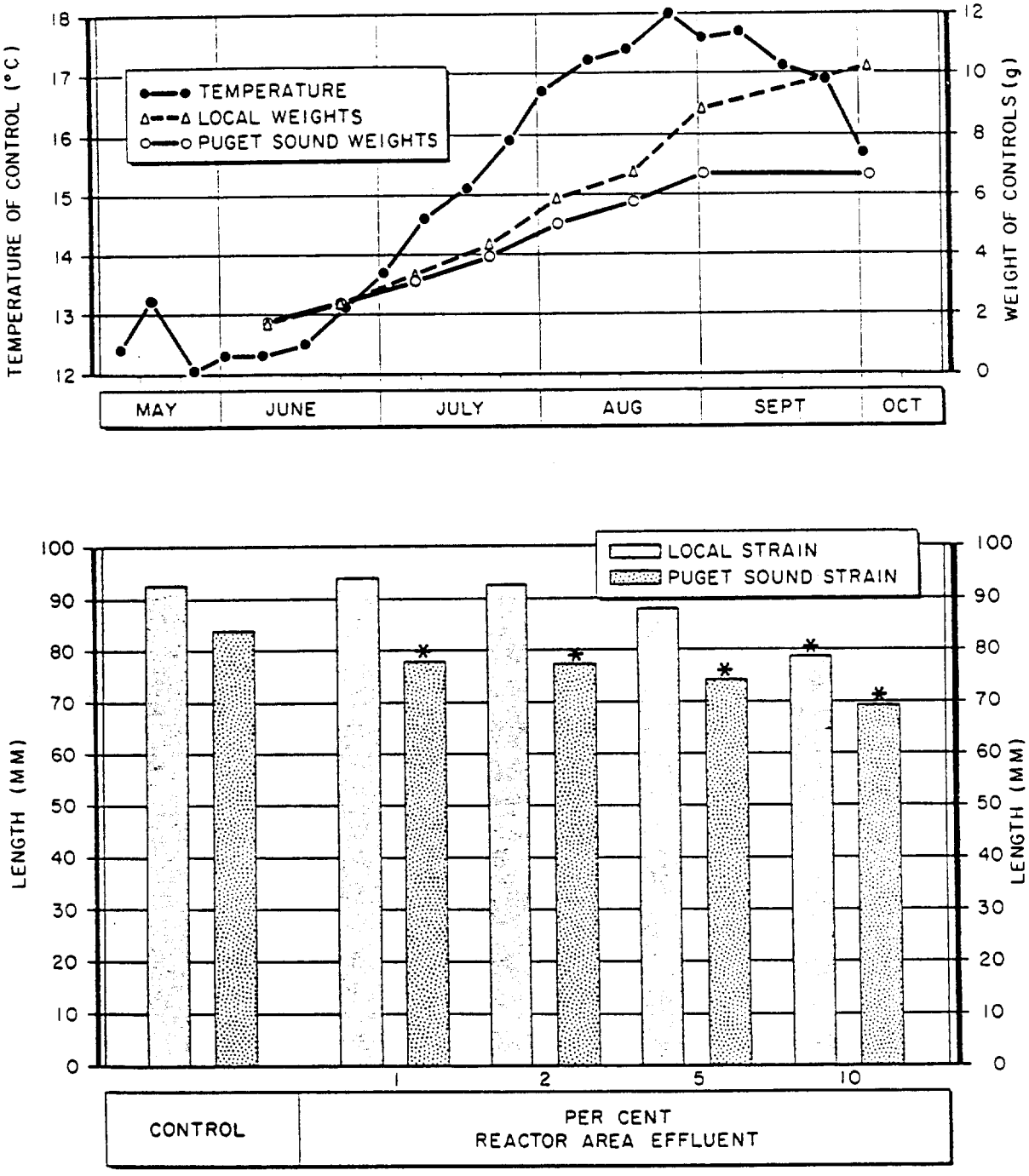

FIGURE 2
GROWTH OF CHINOOK SALMON FINGERLINGS IN VARIOUS CONCENTRATIONS OF REACTOR AREA EFFLUENT

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EFFECT OF REACTOR AREA EFFLUENT WATER ON MIGRANT JUVENILE BLUEBACK SALMON

P. A. Olson and R. F. Foster

Young blueback salmon, typical of those which migrate past the Hanford reactors en route to the ocean, were exposed to dilutions of reactor area effluent water for over three months. No adverse effects were observed even at the 10 per cent level, the most concentrated mixture tested. Since young blueback salmon in the Columbia River would experience much weaker concentrations for much shorter periods of time, no hazard from the effluent is visualized for this species.

The blueback salmon, Oncorhynchus nerka, is one of the most commercially valuable species in the Columbia River. With very few exceptions they spawn in areas a considerable distance upriver from the Hanford plant (1). The adults swim past the reactors en route to their spawning grounds. After the young hatch they usually spend one full year in freshwater lake systems and migrate to the ocean during the spring of their second year. The young fish migrate past the reactors on their way to the ocean. This study was designed to determine the tolerance of migrant-sized blueback salmon to various dilutions of the reactor area effluent.

METHODS

Ninety-two blueback salmon*, weighing about 25 g each, and approximately 18 months old, were placed in each of four concrete ponds of 90 cu ft capacity. The following concentrations of reactor area effluent water were supplied at the rate of 15 gpm.

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* Obtained through the courtesy of the Fish and Wildlife Service, Winthrop, Wash.

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Pond 1	Columbia River Water (Control)
Pond 2	2.5% Reactor Area Effluent
Pond 3	5% Reactor Area Effluent
Pond 4	10% Reactor Area Effluent

(Reactor area effluent is predominantly reactor coolant but also contains small amounts of water from other industrial sources.)

The fish were given normal fish-cultural care. Each lot was weighed at the start of the experiment and the individual weights of the surviving fish were taken at the end of the test. The experiment was terminated late in July of 1954, well after the time when most of the migrants are presumed to have passed Hanford. The exposure of more than three months is several times longer than these fish would ordinarily remain in this section of the Columbia.

RESULTS AND DISCUSSION

Mortalities, shown in Figure 1, were somewhat greater than normal in all lots owing to a kidney disease not associated with the variables tested. In spite of the somewhat weakened condition of the fish, no significant increase in mortality resulted from the presence of the effluent.

Growth rate is often a more sensitive index of toxicity than is mortality. The average size of the fish in the various lots at the end of the test is shown in Figure 1. Again there was no significant effect attributable to the presence of the effluent water.

This test demonstrates that young blueback salmon of migrant size and age are able to tolerate concentrations of reactor area effluent water as high as 10 per cent for considerable periods of time without excess mortality or retarded growth. Since concentrations of the effluent in the Columbia River ranged well below all experimental levels, it seems reasonable to conclude that the effluent from the Hanford reactors had no detectable effect on young blueback salmon migrating past the Hanford reactors.

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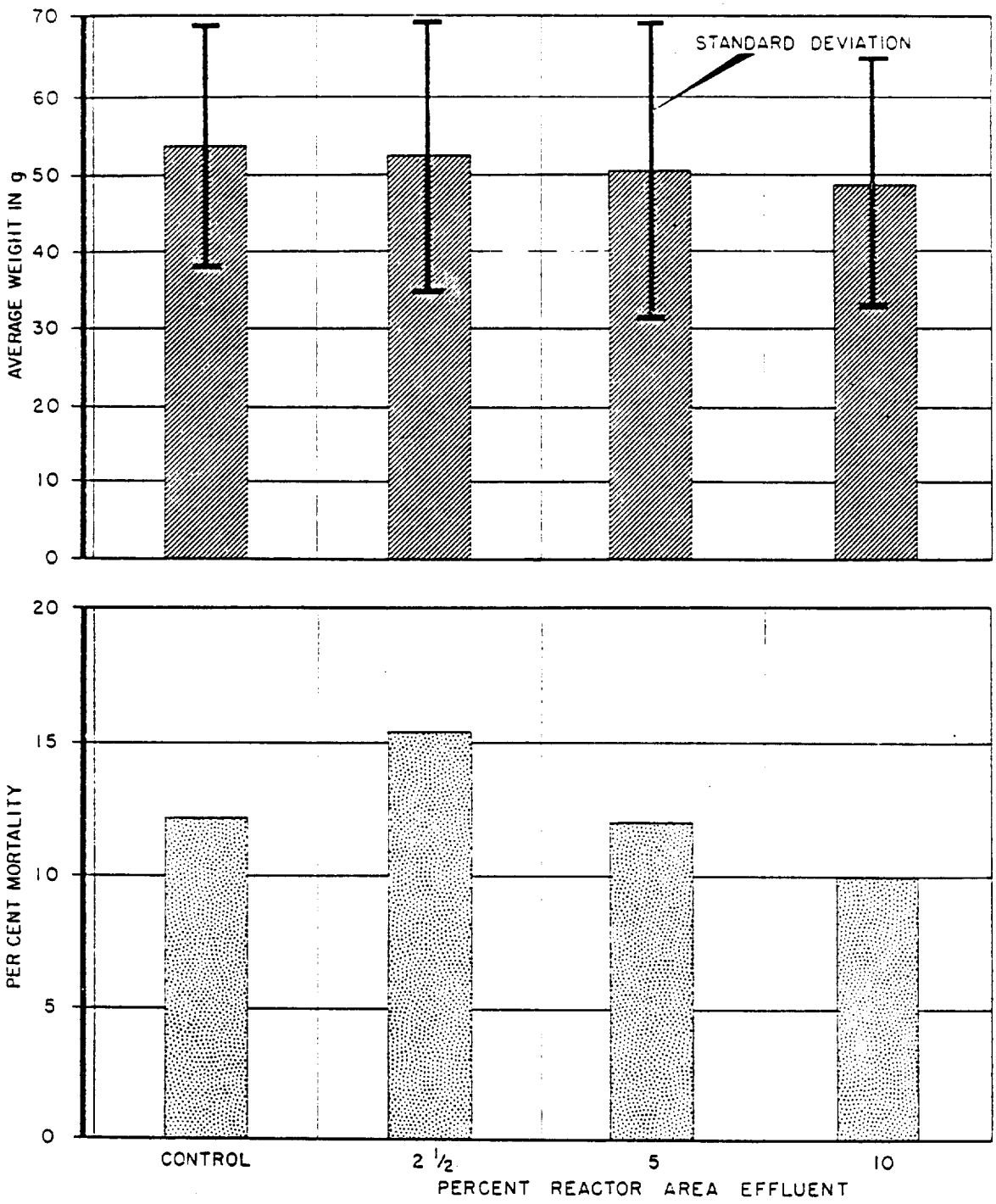

FIGURE I
MORTALITIES AND GROWTH OF BLUEBACK SALMON IN
VARIOUS CONCENTRATIONS OF REACTOR AREA EFFLUENT

AEC-GE RICHLAND, WASH

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ACKNOWLEDGMENTS

Thanks are extended to A. C. Schroeder and the Biological Attendants who assisted with the care of the animals, operation of equipment and recording of data. Statistical analyses were made by Radiation Measurement Evaluation Unit of the Biophysics Section.

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CHRONIC EFFECTS OF REACTOR EFFLUENT WATER ON CEREAL PLANTS - 1954

J. F. Cline, A. A. Selders, and J. H. Rediske

The effects on barley of irrigation with various dilutions of reactor effluent are described. Seed yield is significantly decreased by watering with 100 per cent effluent. No significant effects resulted from watering with five per cent effluent.

This report presents the third year's data obtained from the experiment studying the chronic effects of reactor effluent water on cereal plants. Data for the first and second year were reported in the previous Annual Reports (1, 2).

METHODS

Seed obtained from the 1953 barley crop was replanted in the same plot from which it was harvested. Methods of watering, harvesting, and the analytical procedures were generally the same as those previously reported (1). A change was made in the method of collecting the water samples for spectrochemical analysis. Whereas previously the sample was taken from a single irrigation, this year a composite sample was obtained by pooling one liter of water from each irrigation. The composite sample was submitted for analysis at the end of the season. The plots were watered nine times during the year for a total water application of 10 milli-acre inches.

The seedling mutation rates of the 1952 and 1953 seed were determined by the methods of Smith (3). Tests were made on 4,000 seeds from each plot. The seedlings were grown in a greenhouse to a height of approximately 5 inches, at which time the mutants were counted.

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Yields of seed (Table 1) were less at the 100 per cent level than in 1953 and were significantly less than the yield from the control and five per cent plots. Yield of vegetation seems to vary with yearly environmental fluctuations, while seed yield tends to be stable. As in previous years, only the plots treated with 100 per cent effluent differed from the control plots. Activity densities of the seeds were comparable to those found in previous years, but the activity densities of vegetation were lower than in 1953 and more comparable to those of 1952. The activity in vegetation from Plot IV (five per cent effluent) seems to be out of line for no apparent reason.

The nitrogen content of seeds for all plots was slightly higher than last year. There was no reduction of nitrogen in the 100 per cent effluent treated plots as was found in 1953.

The 1952 and 1953 seed showed no increased frequency of mutations in the treated plots as compared with the controls.

ACKNOWLEDGMENT

The authors are indebted to R. W. Brosemer for nitrogen analyses.

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TABLE 1

Summary of Results from Effluent-Watered Plots - 1954

Factor Tested	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)	1538	1830	1651	1498	1466	1061
Harvest Weight of Grain (g)	1447	1530	1410	1310	1115	1073
Activity Densities of Vegetation (μc/g)	1.5×10^{-5}	2.0×10^{-5}	1.8×10^{-5}	3.8×10^{-5}	1.8×10^{-5}	3.1×10^{-5}
Activity Densities of Grain (μc/g)	2.5×10^{-5}	1.5×10^{-5}	1.6×10^{-5}	2.2×10^{-5}	3.0×10^{-5}	3.1×10^{-5}
Total Seed Nitrogen (mg N/g)	20	19	18	22	24	22
Activity Densities of Soil (μc/g)	2.6×10^{-5}	2.7×10^{-5}	1.9×10^{-5}	1.7×10^{-5}	1.8×10^{-5}	2.7×10^{-5}
Germination (Per Cent Viable)	100	100	100	100	100	99

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CHROMIUM TOXICITY IN PLANTS

J. H. Rediske, J. F. Cline, and A. A. Selders

The effect of chromium concentration on several plant functions is described. The concentration of sucrose, reducing sugars and starch are more affected than the concentration of protein. Chromium(VI) is much more toxic than is chromium(III).

Porter and Cline (1) have reported a chlorotic effect produced in plants by the dichromate ion. The present investigation was undertaken to determine the levels at which chromium, as a cation and as an anion, may be toxic to plants and the specific symptoms produced.

METHODS AND RESULTS

A preliminary survey was conducted with bean plants grown in nutrient solution according to techniques previously described (2). As shown in Figure 1, even very low concentrations of chromium were effective in reducing the dry weight of the leaf portion. The roots were likewise affected, but not as extensively as the leaves. Since dry weight production is related to growth, it is obvious that even concentrations of chromium(VI) as low as 0.01 μg/ml exert a toxic effect in nutrient culture.

The effect of the two valence states of chromium on the starch concentration of bean leaves is plotted in Figure 2. As the chromium concentration increased there was an initial increase, followed by a decrease, in starch concentration. The effect was most pronounced with Cr(VI). Cr(III) was less toxic by at least a factor of 10.

Additional effects of chromium on carbohydrate metabolism are evidenced in Figure 3, which shows the effects on sucrose and reducing sugar concentrations. With increasing chromium the concentration of the

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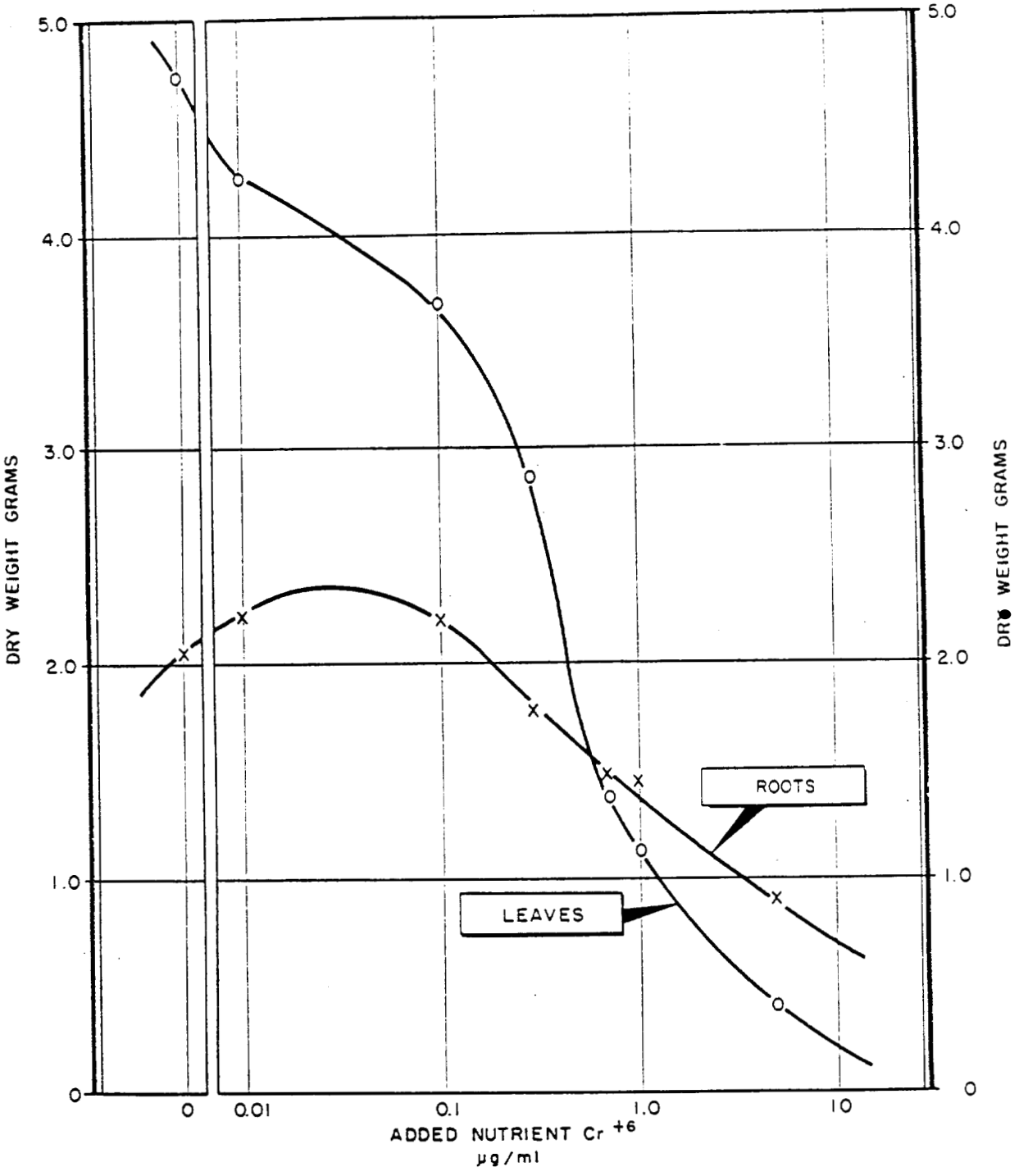

FIGURE 1
THE EFFECT OF VARIOUS CONCENTRATIONS OF Cr (VI) ON THE DRY WEIGHT PRODUCED BY BEAN PLANTS IN NUTRIENT SOLUTION CULTURE

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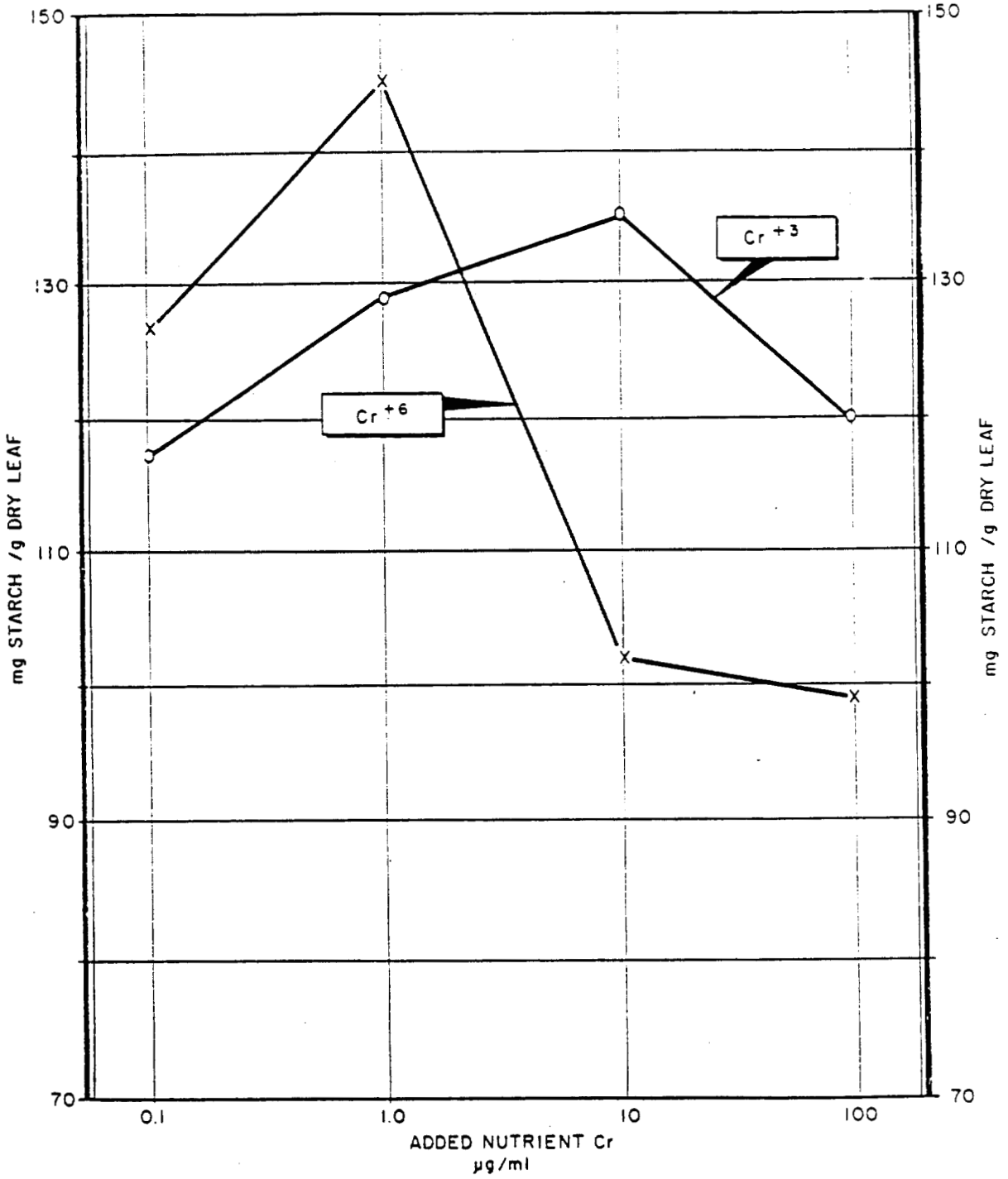

FIGURE 2

STARCH CONCENTRATION IN BEAN LEAVES GROWN IN NUTRIENTS CONTAINING DIFFERENT CONCENTRATIONS OF Cr(III) OR Cr(VI)

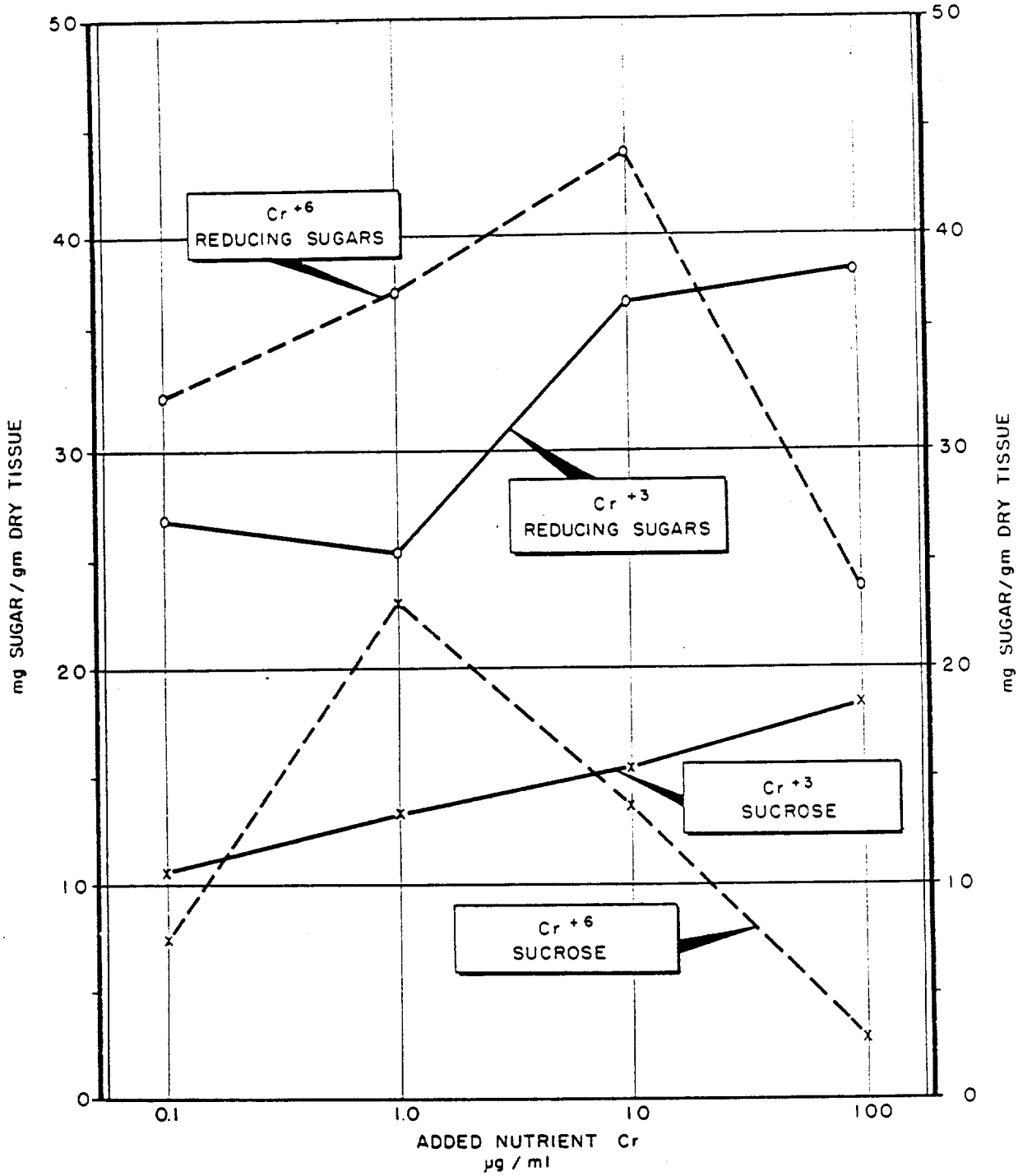

FIGURE 3

THE EFFECT OF Cr (III) AND Cr (VI) CONCENTRATION ON SUCROSE AND REDUCING SUGAR CONCENTRATION IN THE LEAVES

sugars tested first rose and then rapidly fell. Sucrose seems more sensitive than the reducing sugars, since the sucrose reached a peak concentration at lower chromium concentrations than did the reducing sugars. Again, the Cr(III) was less toxic than Cr(VI).

There was little effect of chromium on the protein nitrogen, especially in the leaves (Figure 4). There appears to be a decrease in protein nitrogen in the roots at the highest concentration tested of both valence states of chromium. However, when Cr(VI) was added to soil in which barley plants were grown to maturity, it was found that the protein nitrogen content of the grain increased by about eight per cent over the controls (Figure 5). The chromium was added to the top surface of the soil in amounts up to 180 mg/ft^2 of soil. The outward evidence of toxicity by this much chromium is a delay of several days in the seed germination. This is followed by a generally retarded development and as much as four weeks' delay in maturity.

DISCUSSION

These preliminary data indicate that Cr(VI) is quite toxic to plants. Along with the outward signs of toxicity there are also metabolic disturbances. Carbohydrate metabolism is apparently more sensitive than other phases of plant activity. This is probably associated with the severe chlorosis resulting from chromium toxicity. Since sucrose concentration reaches a peak before the reducing sugars do, the enzymatic conversion from sucrose to glucose appears to be particularly sensitive to chromium in the carbohydrate metabolic sequence. The effect on carbohydrate metabolism is again reflected in plant growth (dry weight production), which is appreciably curtailed as the Cr(VI) concentration increases.

Chromium(III) is considerably less toxic than chromium in the higher valence state, and is probably no more toxic than other similar metals.

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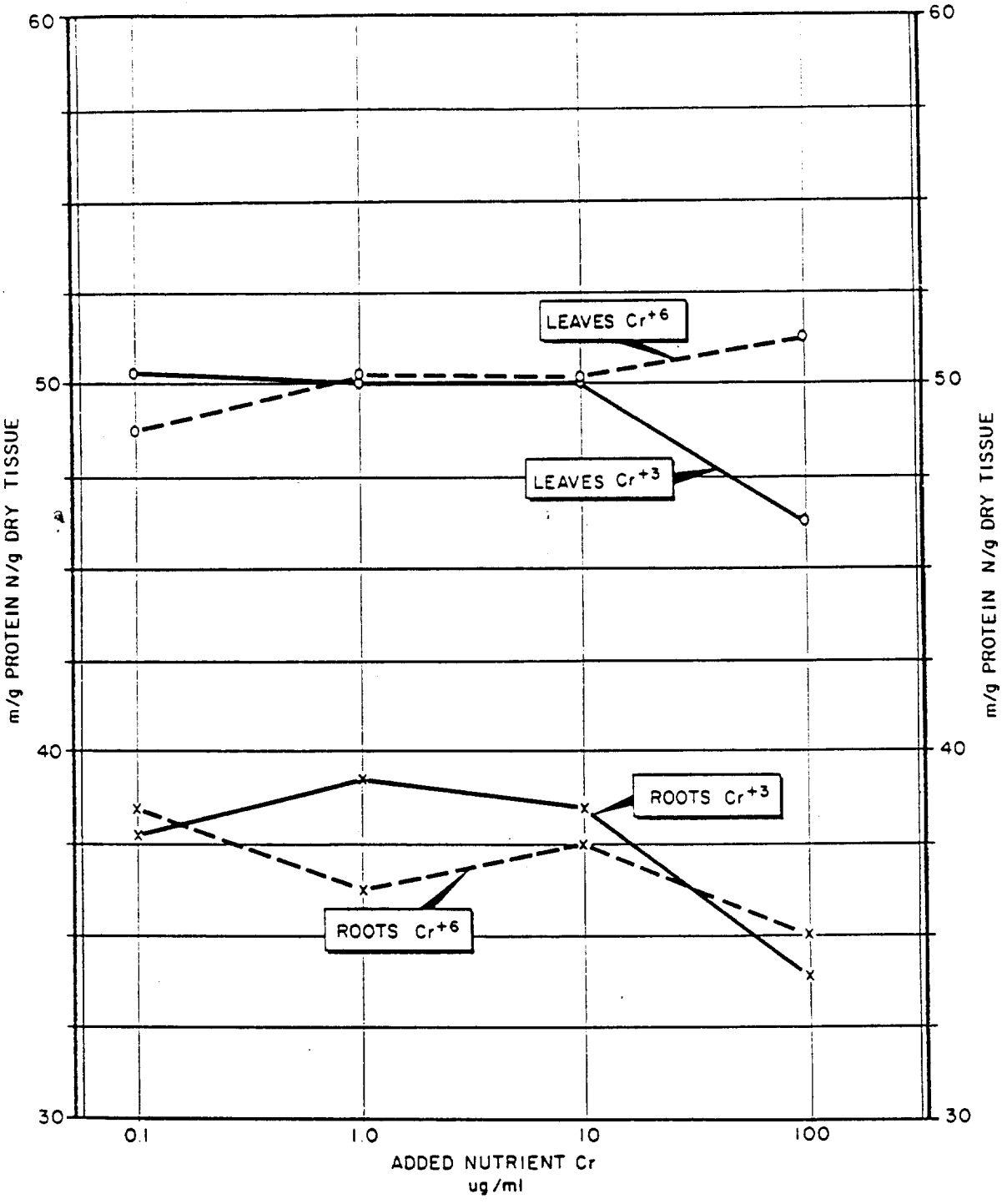

FIGURE 4

THE EFFECT OF CHROMIUM CONCENTRATION ON THE PROTEIN NITROGEN CONCENTRATION OF LEAVES AND ROOTS OF BEAN PLANTS

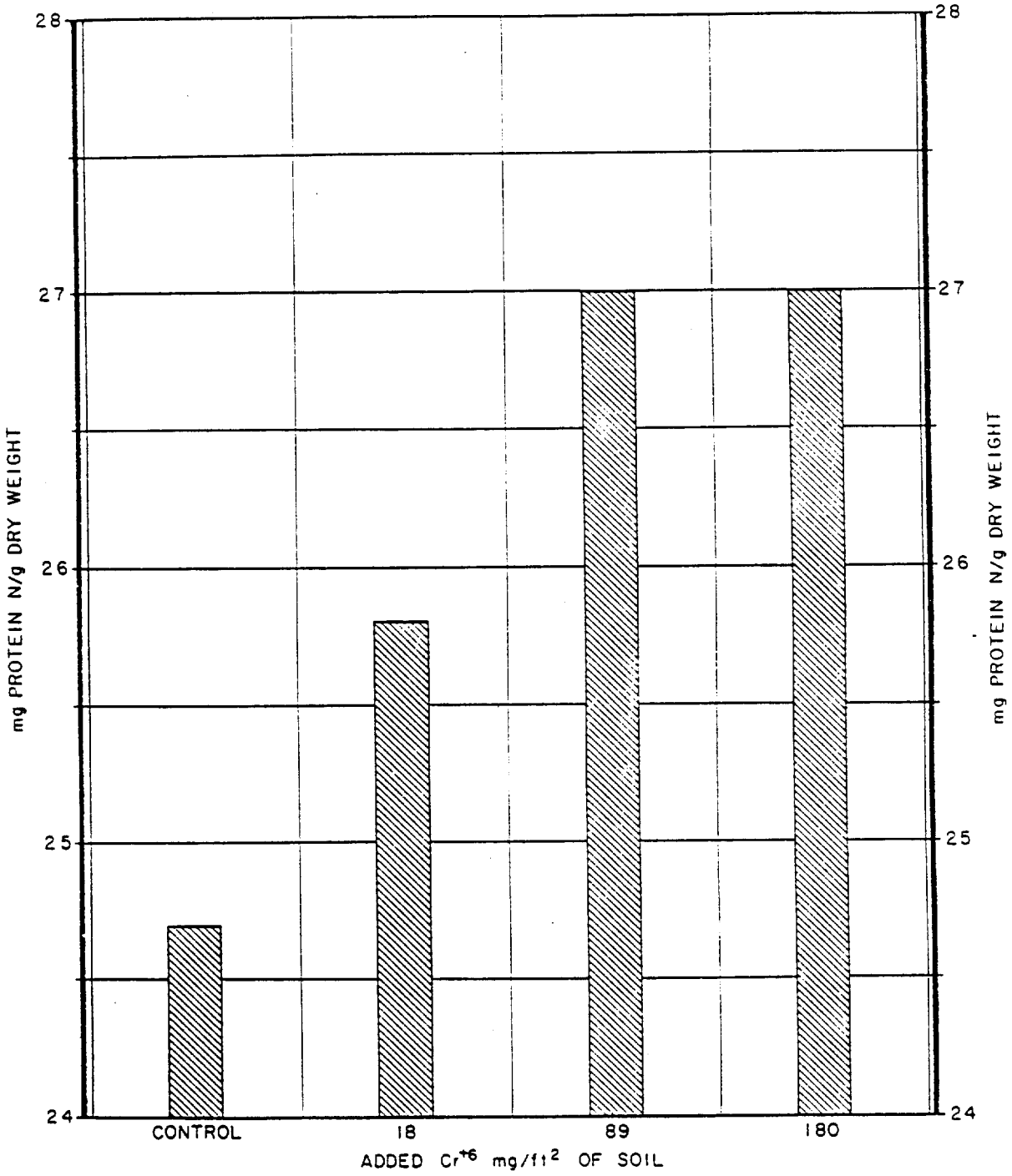

FIGURE 5

THE SEED PROTEIN NITROGEN CONCENTRATION AS INFLUENCED BY CHROMIUM ADDED TO THE SURFACE OF THE SOIL BY IRRIGATION

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ACKNOWLEDGMENTS

The authors are indebted to R. W. Brosemer and R. J. Morrow for the many analyses performed in this work.

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THE ABSORPTION OF FISSION PRODUCTS BY PLANTS

J. H. Rediske, J. F. Cline, and A. A. Selders

Concentration factors for nine fission products and plutonium, as determined on nine local soils, indicate that the radioisotopes of strontium are potentially the most hazardous to agriculture. Results of studies of the effect of carrier concentration, pH, plant species, plant organ, and soil on the absorption of fission products are reported.

The purpose of this work was to determine the quantity of these fission products absorbed by plants and some of the factors which may influence this absorption. To facilitate comparisons between isotopes the results are expressed as a concentration factor which is the ratio of the concentration of the fission product in the dried plant matter to its concentration in the root environment.

METHODS

Uptake from soil was determined by the Neubauer seedling test. The details of this test have been previously described (1). The effect of pH and carrier concentration were studied by standard nutrient culture techniques (1). All the following data are based on dry weights. The fresh weight of most materials discussed herein, with the exception of seeds, is approximately ten times that of the dry weight.

RESULTS

Effect of Soil

The concentration factors for the important fission products for nine south central Washington soils are reported in Table 1. Strontium and iodine have the largest concentration factors, with barium and cesium

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slightly smaller. All other fission products have a concentration factor lower than strontium by 100 or more.

It is apparent from the data of Table 1 that variations in the amount of fission product absorbed from different soils are not in excess of one order of magnitude. Although all the local soils are alkaline, no great difference is found when a comparison is made of these results with those obtained on acid soils (2).

Effect of pH

Figure 1 shows the effect of nutrient pH on the concentration factor for six fission products and plutonium. Within the range studied there is a general increased absorption as the pH decreases. The increased uptake with increased acidity is very evident with yttrium, but barely perceptible with cesium.

Effect of Carrier Concentration

The effect of addition of carrier on the uptake of fission products is plotted in Figure 2. As the carrier concentration is increased in the nutrient environment the concentration factor for a particular fission product tends to increase.

Effect of Organ and Species Differences

The concentration of fission product is as great or greater in leaves than in any other organ. The concentration in bean leaves was compared with that in the pod and seeds, tomato leaves with the fruit, wheat leaves with the head, and russian thistle leaf and stem with the fruit. Variations were seldom greater than a factor of ten when either different organs of one species or different species were compared.

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TABLE 1

Concentration Factors $\left[\frac{\text{Conc. of Isotope in Leaves}}{\text{Conc. of Isotope in Soil}}\right]$ of Several Isotopes as Determined by the Neubauer Seedling Test Using Barley Grown in Representative Soils

Soil	pH	% Organic	Cation Exchange Capacity milli-equiv. /100 g	Concentration Factors for Isotopes Tested									
				Sr^{90}	Ba^{140}	I^{131}	Cs^{137}	Y^{91}	Ce^{144}	Pm^{147}	Ru^{106}	Zr^{95}	Pu^{239}
Ringold Silty Clay	7.6	7.9	34.1	.96	.13						.0083		
Wheeler Silt Loam	7.7	4.3	4.8	1.6			.037		.0041		.012	.019	
Ritzville Very Fine Sandy Loam	7.8	3.0	5.2	1.2									
Sagemoor Very Fine Sandy Loam	8.2	2.8	7.1	1.0							.0097		
Ephrata Loamy Sand	7.3	1.8	8.1	1.7		1.05	.10	.006			.015		.0009
Warden Loamy Very Fine Sand	7.7	2.7	6.0	2.5							.0098		
Ephrata Fine Sandy Loam	7.5	1.9	4.4	2.0							.012		
Quincy Sand	7.3	2.0	3.5	4.5									
Winchester Fine Sand	7.4	2.4	3.4	3.0	.32		.076		.0038	.0013	.012	.0039	

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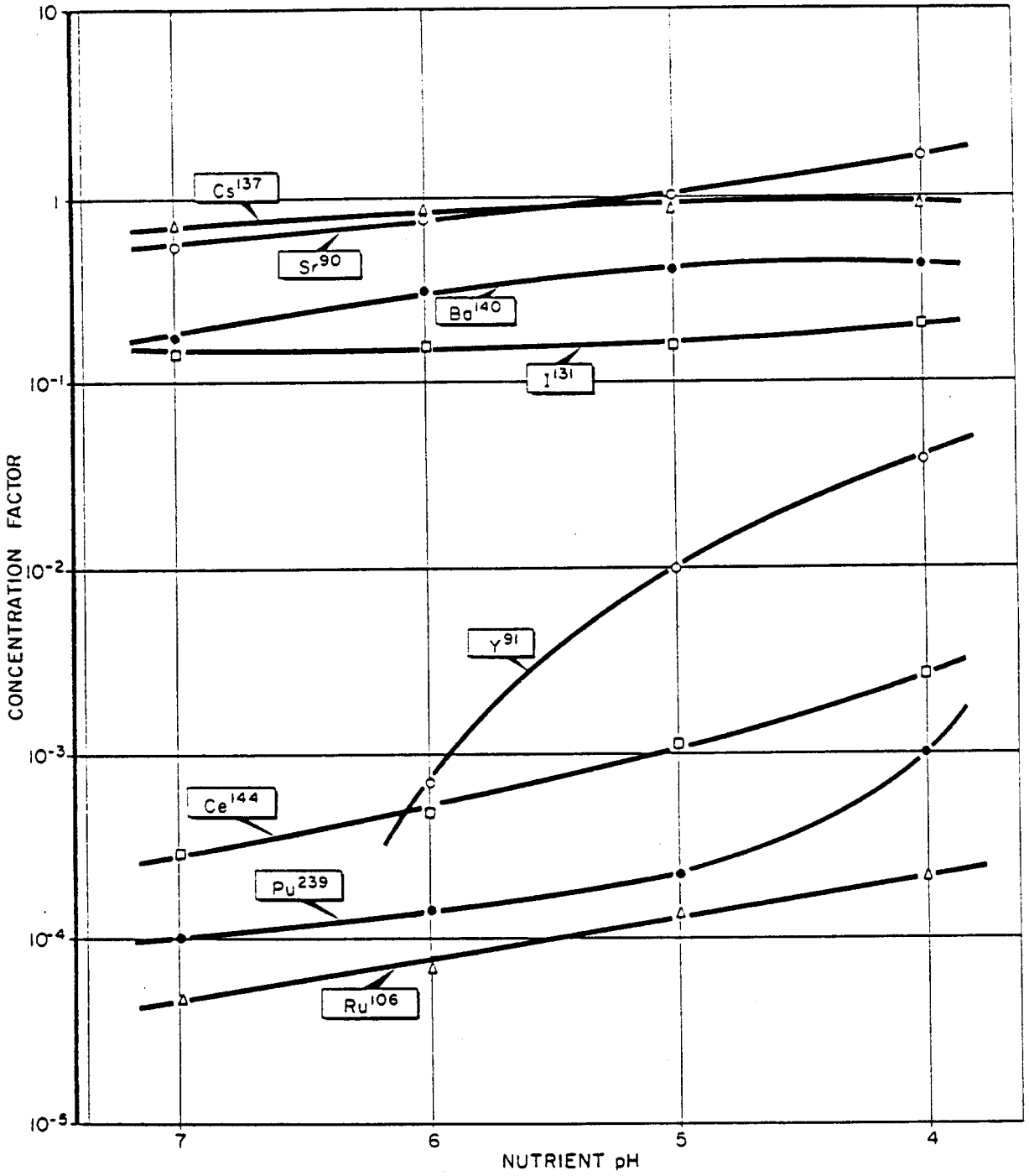

FIGURE I
THE EFFECT OF pH ON THE CONCENTRATION FACTOR

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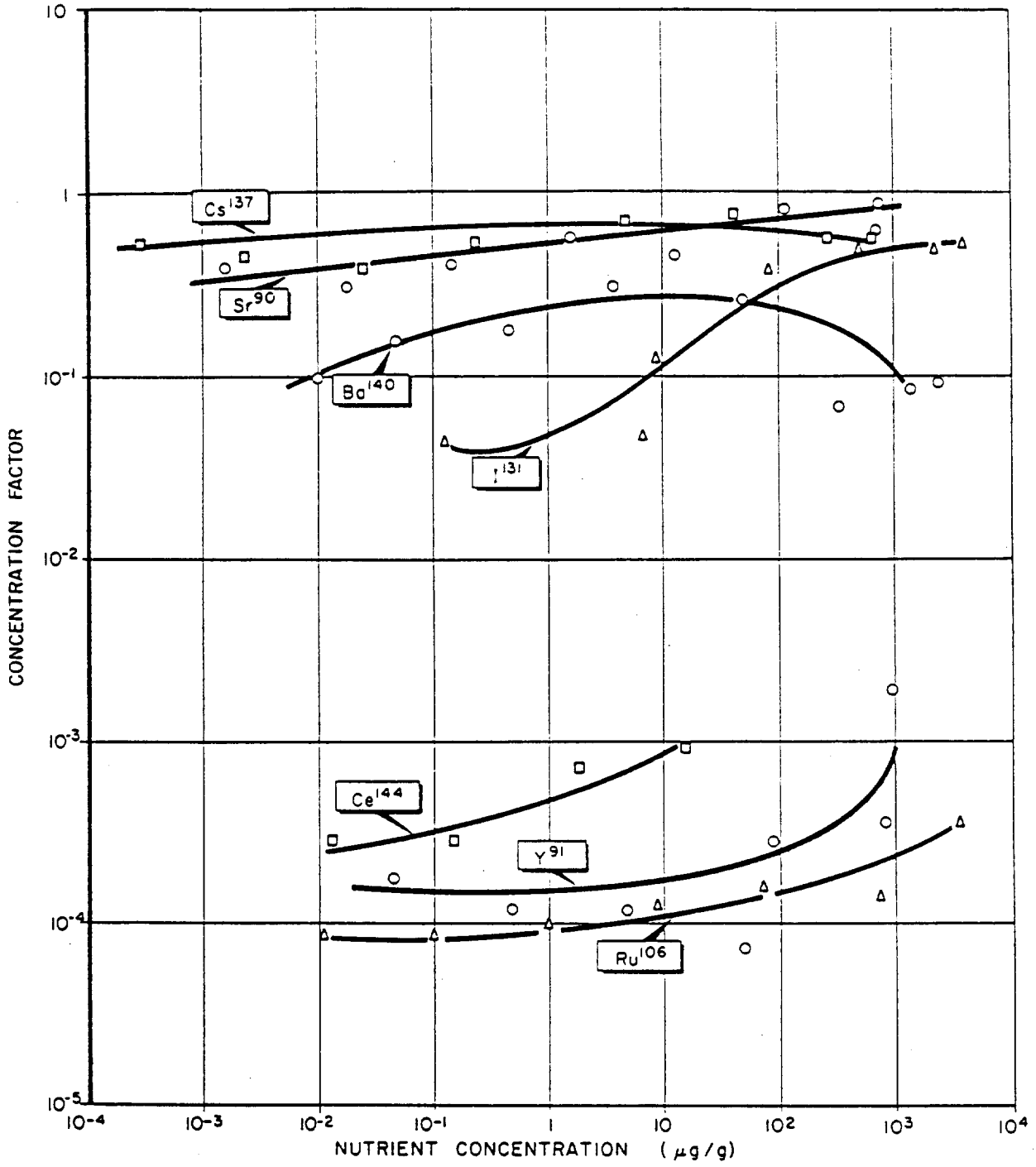

FIGURE 2
THE EFFECT OF CARRIER CONCENTRATION ON THE CONCENTRATION FACTOR

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DISCUSSION

Concentration factor is used in expressing the data because this factor derives from the concentration of the isotope in the root environment. In the soil, where the substrate is essentially limitless, the concentration of the isotope in the root area governs the amount absorbed by the plant. Likewise in nutrient solution culture the concentration of element adsorbed onto colloids in the immediate vicinity of the root is much more important than the amount free in solution (1). Soil or solution studies are thus more intercomparable when the concentration factor is used to express results.

The Neubauer technique for finding concentration factors usually yields results which are greater than those obtained from field tests, because of suboptimum growing conditions and uneven isotope distribution in the field. Tests in nutrient solution supplement the soil tests and may allow extrapolation to soils other than those listed. However, caution should be exercised in so applying these data. For example, in using the data on pH (Figure 1), characteristics of the soil may outweigh a pH effect. Particle size, amount of organic matter, and other soil factors exert their own effect, and if these depress uptake the pH effect may not be apparent. Thus if data derived from nutrient solution studies are used to modify concentration factors obtained from soils, they should not be considered absolute determiners of what to expect from different soils.

Of the several factors evaluated for their effect on absorption, all produced variations generally less than one order of magnitude. No attempts have been made to determine the additivity of these environmental influences.

From the concentration factors and the half-lives, it is apparent that the isotopes of strontium are the most hazardous fission products when absorption into crops is considered. Ba^{140} and Cs^{137} are slightly less important than strontium. I^{131} is of consequence for only a short time following fission.

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That isotopic dilution of the radioactive isotope with non-radioactive isotope did not decrease uptake is apparent from Figure 2. This lack of effect of isotopic dilution applied to all elements tested at concentrations that were non-toxic. However, when concentrations of carrier were increased to the point of producing severe toxic reactions in the plants, a depression of uptake was noted (3).

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ABSORPTION OF STRONTIUM INTO PLANTS FROM FIELD PLOTS

J. F. Cline

The uptake of Sr^{90} by barley from surface contaminated soil was measured. No apparent differences were observed in strontium uptake by barley between surface and sub-surface irrigated plots.

This experiment was designed to relate values obtained from Neubauer studies with those from field conditions.

METHODS

Twelve milliacre plots, each measuring 4 by 10 feet, were constructed from plastic-painted planks which were sunk into the soil leaving three inches above the ground level. Each plot was tilled, 169 g of fertilizer (analysis 16-20-0) were added, and 145 g of a pure strain of Himalayan barley seed were added. Following germination the soil surface was uniformly contaminated with one millicurie of Sr^{90} per plot by means of a sprinkling device. Areas of ground between the sprouted barley were covered with strips of cheese cloth to prevent the spread of contamination by the wind.

Irrigation water was distributed by canvas soakers onto the surface of irrigated plots, and through a buried perforated plastic hose for the sub-surface irrigated plots. All plots were irrigated six times during the growth period (13 weeks), for a total water application of 12 milliacre inches.

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The Sr^{90} concentrations in vegetation and seed were determined by standard counting technique after the samples were digested in nitric acid and dried.

Soil samples were taken to determine the distance that the Sr^{90} percolated into the soil by the end of the season. The samples were obtained by digging a hole with one sheer side. At specified levels a 1/8-inch layer of soil was removed from the sheer surface for analysis.

RESULTS AND DISCUSSION

Table 1 indicates that no significant difference occurred in the uptake of strontium by barley grown in the surface as compared to sub-surface irrigated plots.

TABLE 1

Uptake of Sr^{90} into Barley from Contaminated Soil
(μc/g dried* sample)

	Type of Irrigation	
	Surface	Sub-Surface
Vegetation	1.3×10^{-3}	8.9×10^{-4}
Seed	6.4×10^{-4}	1.8×10^{-4}

* Dried at 76° C for 12 hours. Approximate water content of vegetation, 90 per cent, and of seed, 10 per cent.

Soil samples taken from the surface-irrigated plots at the end of the growing season showed the following distribution of Sr^{90}: 96 per cent in the top 1/8-inch layer, 3.7 per cent at 1/4 inch, 0.2 per cent at one inch,

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and 0.02 per cent at three inches. Apparently very little strontium penetrates beyond the surface layer of soil. No samples were taken from the sub-surface irrigated plots.

Seed germination tests showed that there was no effect of the Sr^{90} on the viability of the seed. The uptake of Sr^{90} by the barley in the plots was approximately 0.03 per cent of the total amount applied to the soil. This is considerably less than the 4 per cent uptake found in Neubauer tests (1). The large difference between the field and Neubauer percentages can be partially explained by the fact that the activity in the plots was almost totally located in the top quarter inch, while it was uniformly distributed throughout the soil in the Neubauer tests. Thus, only a small portion of the root system was exposed to the Sr^{90} in the field test, whereas all roots were exposed in the Neubauer tests. Since the plots were not ready for planting until late in the growing season, when abnormal growing conditions existed, these results should be considered preliminary.

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GASTRO-INTESTINAL ABSORPTION, DISTRIBUTION, AND RETENTION OF CESIUM FED CHRONICALLY IN VARIOUS FORMS TO RATS

M. H. Weeks and W. D. Oakley

The absorption of cesium from the G. I. tract of rats was not affected by the form in which it was fed, i.e., inorganic solution, mixed with plant material, or biologically incorporated in plant material. Greatest accumulation of cesium was in muscle. Evidence was obtained for the existence of cesium binding components of longer half-life than previously demonstrated.

The metabolism of cesium in a variety of animals has been recently studied by Hood and Comar (1), who also review the earlier literature. By all accounts cesium is virtually 100 per cent absorbed from the G. I. tract. It was the purpose of the present study to determine whether such absorption might be affected by the form in which the cesium was fed.

METHODS

Cesium-137 was administered by stomach tube, daily, for 18 days. Three types of material were administered: 1) a solution of the isotope in 0.9 per cent saline, 2) the same solution plus homogenized leaves from red kidney bean plants, and 3) a suspension of homogenized bean leaves from plants grown in nutrient solution containing Cs^{137} (2). The same volume (1 ml) was fed in each case, and contained approximately 0.2 μc Cs^{137}. Animals employed were adult females of the Sprague-Dawley strain, weighing 235 ± 10 g. The experiment started with 29 rats receiving the solution and 17 rats receiving each of the leaf suspensions. Animals were sacrificed in groups of three during the feeding period. Twenty-four hours after the last feeding, five animals were sacrificed. In addition, rats which

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had received the solution were sacrificed in groups of two at intervals up to 21 days following the last feeding. Urine and feces were collected from five animals in each feeding group from the initiation of feedings until the last animals were sacrificed.

To provide comparable data from acute exposure, excretion of Cs^{137} in urine and feces was followed in three rats for a period of 21 days following feeding of a single dose of the isotope.

Samples taken for analysis were digested with nitric acid and aliquots of the solution counted with an end-window G. M. tube.

RESULTS AND DISCUSSION

Excretion curves during the course of the feedings are shown in Figure 1. Tissue Cs^{137} concentrations attained at the end of the feeding period are shown in Table I, together with figures for the Cs^{137} retained 21 days after the end of the feeding. Excretion curves obtained following feeding of a single dose of Cs^{137} are shown in Figure 2.

Absorption of cesium from the G. I. tract is not affected by its state of incorporation in plant material. This is indicated by the practically identical excretion curves of Figure 1, the low initial excretion in the feces, and the practically identical tissue concentration attained at the end of the feeding period (Table 1).

Distribution of the isotope after the first day's feeding was almost identical with that reported by Hood and Comar (1). Build-up of Cs^{137} continued throughout the feeding period with no distinct leveling off except in the case of bone. The loss of cesium from the tissues during 21 days following the feeding period could not, for most tissues, be represented by a single exponential function. Over this 21-day period the cesium content of

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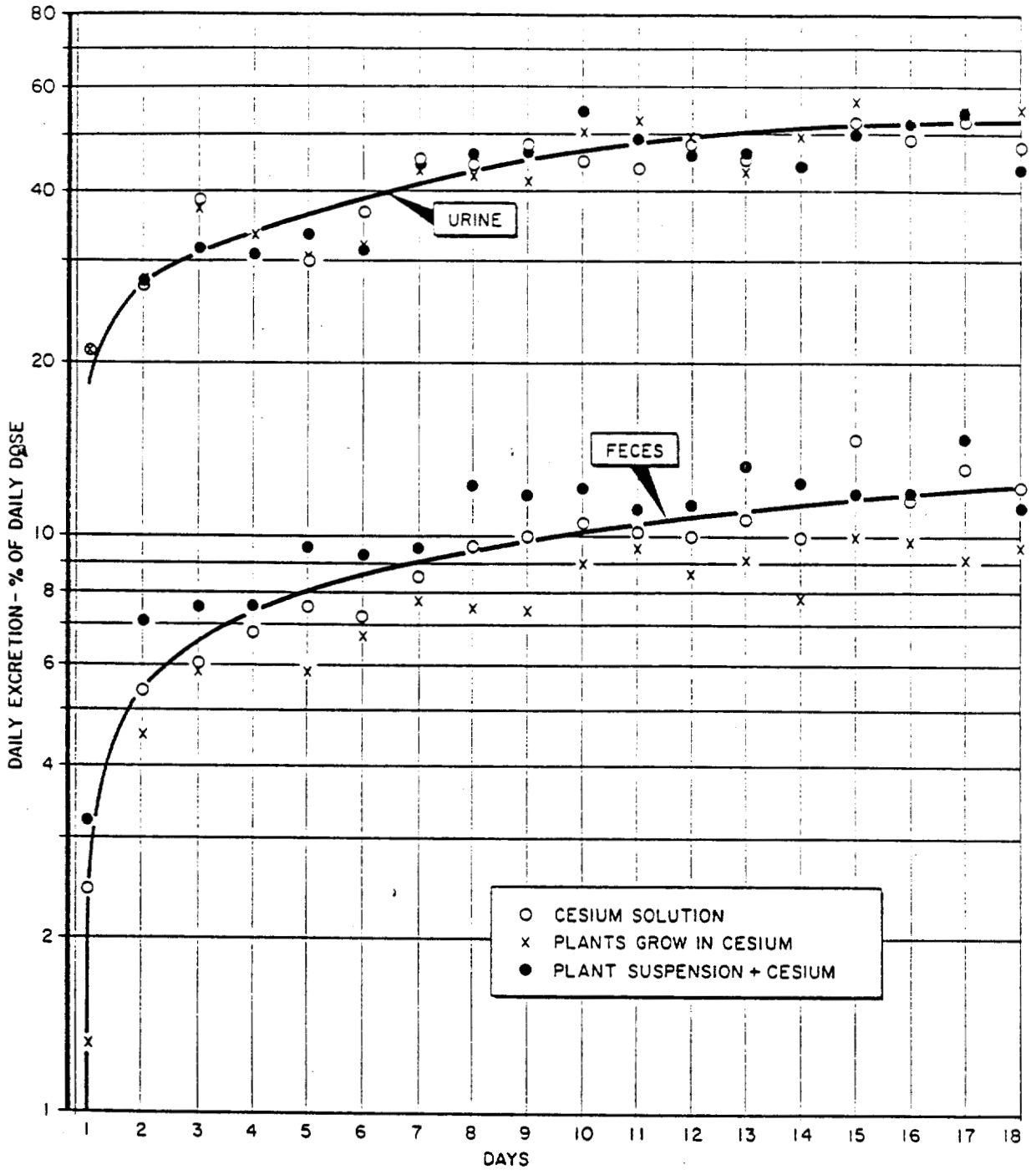

FIGURE I
CESIUM EXCRETION DURING FEEDING PERIOD

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TABLE 1

Distribution of Cs^{137} in Rats Following 18 Days' Chronic Feeding in Various Forms

Organ or Tissue	Concentration in Tissues (Per Cent of Avg Daily Dose/g)			
	At End of Feeding Period			21 Days After Last Feeding
	Inorganic Solution	Inorganic + Plant Material	Incorporated in Plant Mat.	Inorganic Solution
Muscle	5.0	4.6	5.5	1.2
Tongue	3.6	3.3	3.2	0.74
Kidney	3.0	3.0	3.2	0.60
Liver	2.6	2.4	2.6	0.35
Spleen	2.6	1.9	2.1	0.38
Heart	2.2	1.5	1.9	0.39
Lungs	1.7	1.3	1.4	0.29
Brain	1.3	1.0	1.2	0.26
Femur	1.2	1.0	1.1	0.11
Pelt	0.97	0.93	0.86	0.23
Ovaries	0.81	0.76	0.97	0.14
Blood	0.52	0.40	0.54	0.10
	Material Balance (Per Cent of Total Cs^{137} Administered)			
Retained in rat*	38	35	41	10
Excreted in urine	41	41	43	62
Excreted in feces	9.3	10	7.5	14
Total recovered	88	86	91	86

* Estimated from organ and tissue analyses.

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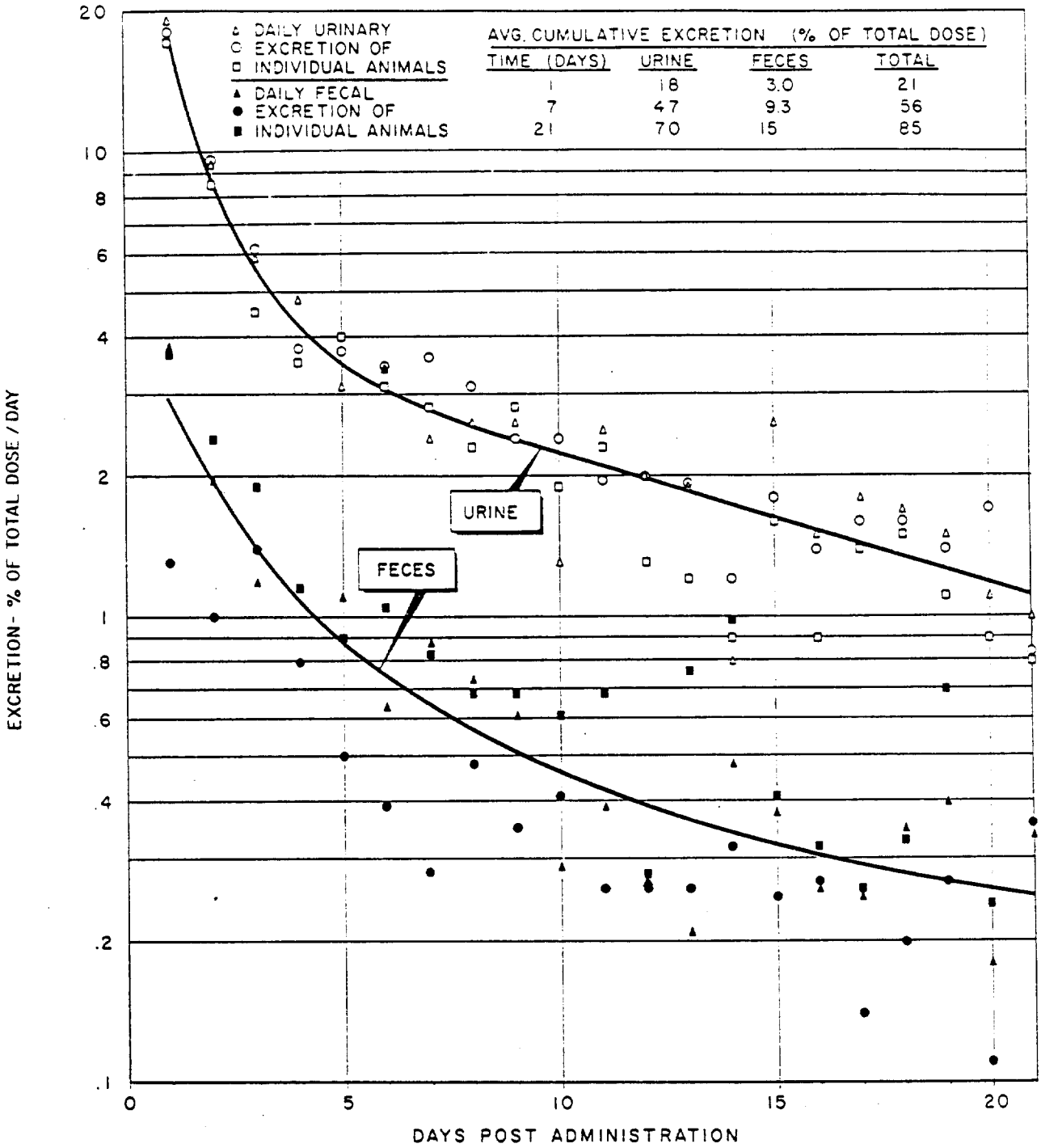

FIGURE 2
EXCRETION OF Cs^{137} BY RATS FOLLOWING SINGLE INTRAGASTRIC DOSE

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most tissues (bone excepted) decreased by a factor of about 5. Hood and Comar, following a single dosing, noted decreases of approximately 10-fold in 14 days and 20 to 100-fold in 28 days (1). The greater retention following chronic feeding would seem to indicate a build-up of cesium in components exhibiting longer biological half-lives than those which initially incorporate the bulk of the cesium following a single administration.

The excretion curves obtained following single intragastric administration of Cs^{137} are similar to those previously reported by Scott, et al. (3), and to excretion curves following intramuscular injection reported by Hood and Comar (1).

ACKNOWLEDGMENTS

We wish to acknowledge the technical assistance of Joan O. Hess and Elizabeth C. Desposato.

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PERCUTANEOUS ABSORPTION OF PLUTONIUM SOLUTIONS IN RATS

M. H. Weeks and W. D. Oakley

The extent of percutaneous absorption of plutonium was not affected by varying the area exposed, or by the presence of a small puncture wound in the exposed area. Absorption increased with acidity of solution applied, possibly due to greater retention at the site of exposure. Little absorption occurred beyond five days of exposure.

Previous studies (1, 2) have been extended in an attempt to define more clearly the various factors which may influence the passage of plutonium through the skin. Two experiments are described, one in which area exposed and acidity of solution were varied, and one in which duration of exposure was varied and animals with and without puncture wounds in the exposed area were employed.

METHODS

All experiments were performed on rats weighing from 250 to 350 grams. The contaminating solution was applied to a defined area in the dorso-lumbar region, which was shaved with an electric animal clipper 24 hours prior to exposure. The contaminated area was not covered or otherwise protected during the exposure period. The contaminating solutions contained Pu(IV) nitrate in nitric acid of varying normality.

At the end of the exposure period animals were sacrificed and the pelt and gastro-intestinal tract were removed and discarded, except for an area of skin about one inch square including the contaminated area. The liver, residual carcass, and contaminated skin area were analyzed for plutonium content using procedures previously described (1).

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RESULTS AND DISCUSSION

Results from the experiment varying acidity and area of exposure are shown in Figure 1. Each group consisted of five rats. Approximately 1 μg Pu^{239} was applied to each animal in a volume of 5 μl and 30 μl respectively for the 0.5 cm^2 and 3 cm^2 areas. All animals were sacrificed after five days' exposure.

Absorption from the smaller exposed area appears to be somewhat greater than from the larger area. The difference is not statistically significant. The variation of absorption with acidity is much more obvious and is significant at the 99 per cent confidence level when absorption is calculated on the basis of plutonium applied to the skin. Recovery of plutonium from the contaminated skin area at sacrifice was 50-60 per cent for the 10 N HNO_3 exposures and only 8-12 per cent for the 0.1 N HNO_3 exposures. It is not clear, therefore, whether the effect of acidity is on the absorption process, per se, or whether the effect is simply one of prolonging the period of contact of plutonium with the skin.

The possibility of puncture wounds increasing the absorption of plutonium through the skin was studied in an experiment in which the duration of exposure was also varied. The wounds were inflicted immediately prior to application of the plutonium solution, using a spring lancet of the type used in the collection of finger blood samples. The wound was approximately 2 mm long and 3 mm deep. Plutonium was applied as 10 μl of a 10 N HNO_3 solution, containing approximately 1 μg, and was spread over an area of 1 cm^2, which included the wound. Results of this experiment are shown in Figure 2.

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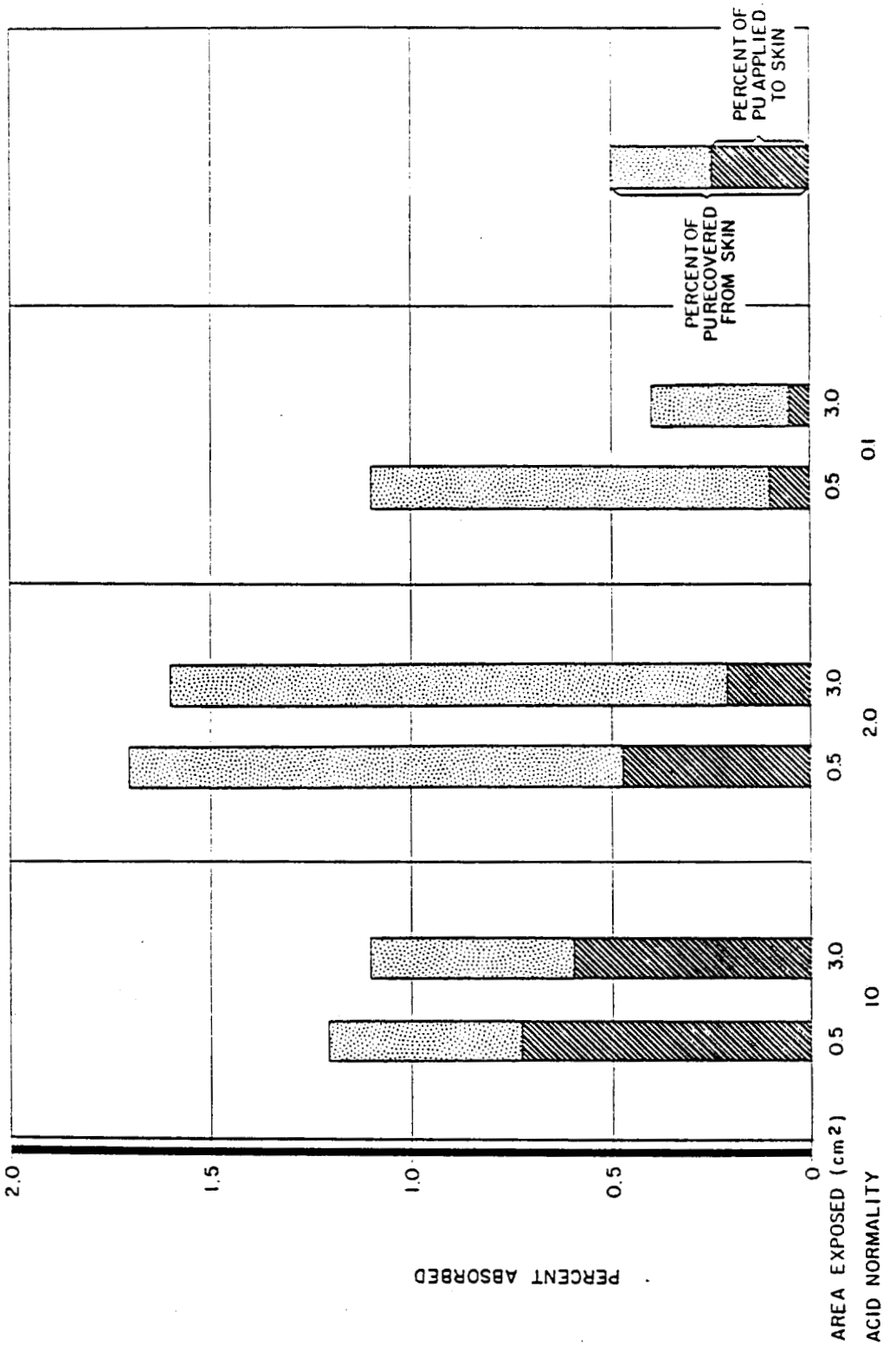

FIGURE 1

EFFECT OF ACIDITY AND EXPOSURE AREA ON PERCUTANEOUS ABSORPTION OF PLUTONIUM

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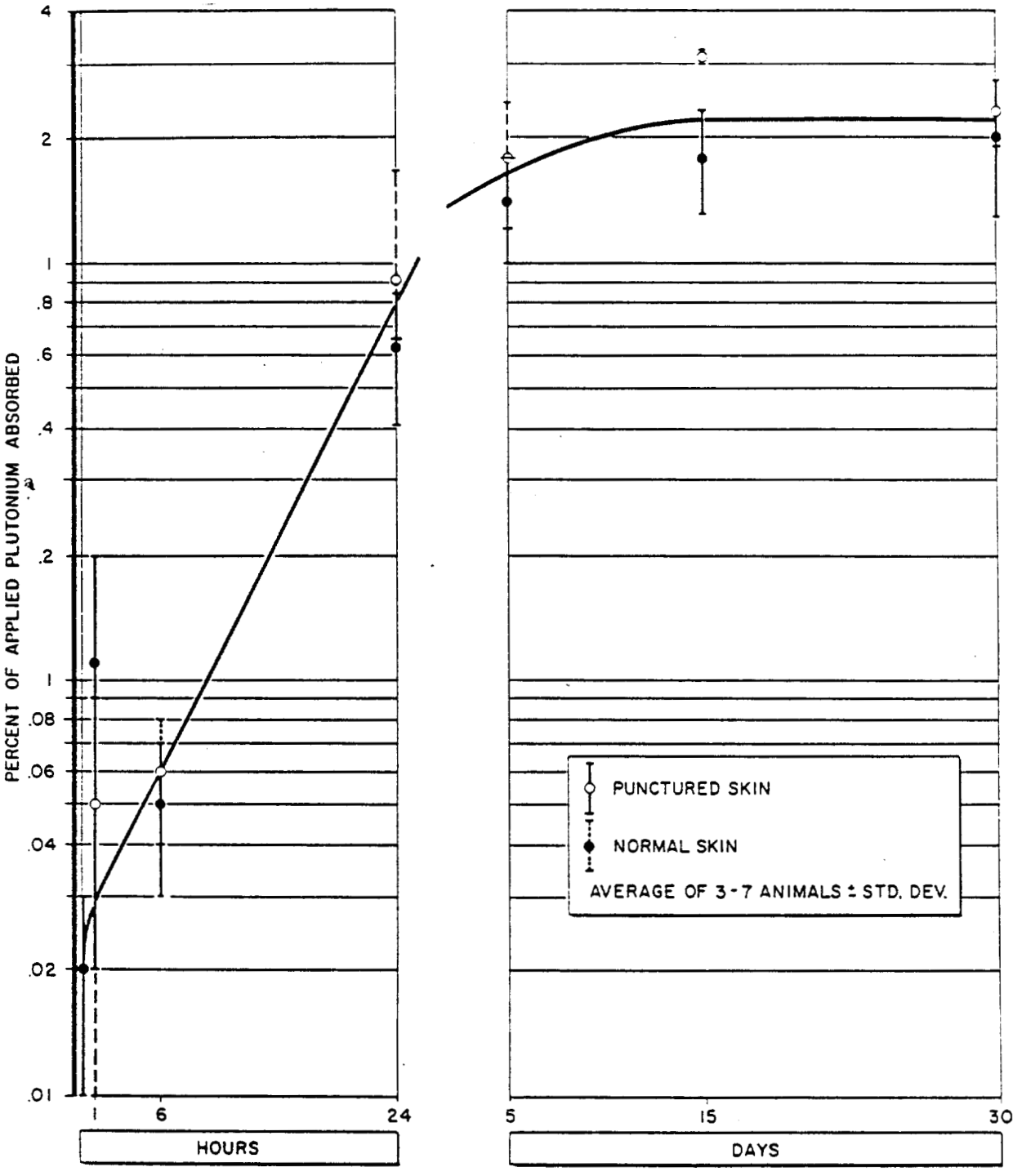

FIGURE 2
EFFECT OF DURATION OF EXPOSURE AND SKIN PUNCTURES ON THE PERCUTANEOUS ABSORPTION OF PLUTONIUM

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Absorption did not continue appreciably beyond the fifth day of exposure, although about 50 per cent of the applied plutonium remained at the site of contamination on the 5th and 15th day. The average maximum absorption of approximately 2 per cent of the applied dose is considerably lower than the figure of 8 per cent obtained in a previous experiment after seven days' exposure (1). In the previous experiment the exposed area was covered with a quick drying, self-sealing fungicidal and bactericidal ointment which apparently caused an increased absorption beyond that attributable to merely holding the plutonium in place. Plutonium lost from the site of contamination and eaten by the animals would not significantly influence results, since only about 0.003 per cent of orally ingested plutonium is absorbed through the gastro-intestinal tract (3).

It is evident that the presence of the puncture wound had no significant effect on the percutaneous absorption of plutonium. It is quite possible that the application of the 10 N HNO_3 effectively cauterized the wound, preventing any absorption through this channel. Histologic examination of skin specimens showed that coagulation occurred through the entire thickness of the skin and the underlying skeletal muscle within 15 minutes after application of the acid. The epidermis surrounding the exposed area gradually undermined the coagulated tissue (Figures 3 and 4), forming a slough which separated between the 15th and 30th day. The final scar at the site of exposure consisted of fibrous tissue covered by regenerated epithelium, with a complete loss of dermal appendages and the coagulated skeletal muscle (Figure 5).

Autoradiographs showed substantial retention of plutonium on the skin surface. Unavoidable spreading of this surface plutonium during processing tends to obscure any true migration of a low order of magnitude. However, definite migration downward along hair follicles was observed

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Figure 3

Early stage in undermining of coagulated tissue by invading epidermis, coagulated tissue occupying left half of area. Specimen obtained three days after exposure. 116 X

Figure 4

Advanced stage of undermining of potential slough with epidermis invading subcutaneous fat layer, seven days after exposure. 38.6 X

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Figure 5

Atrophic skin covered by regenerated epidermis with complete absence of dermal appendages, 30 days after exposure. 116 X

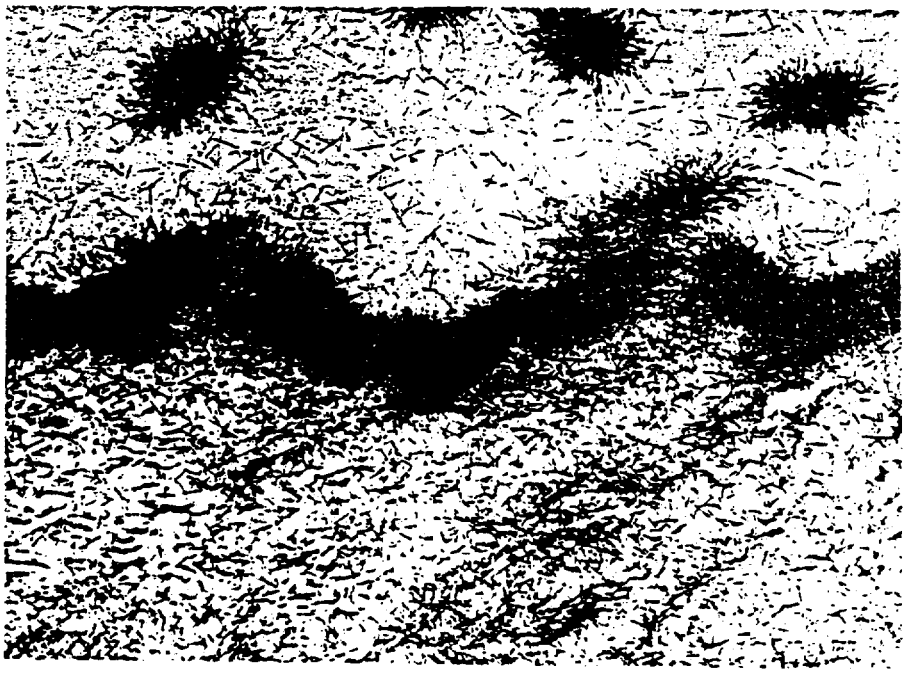

Figure 6

Autoradiograph of skin 15 days after exposure showing dense concentration of plutonium on surface of skin, with migration downward along hair follicles. Focal accumulations of tracks above skin surface are present on hair shafts; general scattering of tracks is considered an artefact. 38.6 X

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at 15 days (Figure 6). Another specimen at 15 days showed a moderate number of tracks in skeletal muscle layer of the developing slough, and at 30 days, after separation of the slough, a few tracks were observed in macrophages in the deepest layer of the atrophic skin.

ACKNOWLEDGMENTS

We wish to acknowledge the technical assistance of Joan O. Hess and Elizabeth C. Desposato in the exposure and analysis of the rats. Histologic and autoradiographic preparations were prepared by N. L. Dockum and interpreted by Dr. S. Marks.

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COMPARISON OF GASTRO-INTESTINAL ABSORPTION OF PLUTONIUM IN THE RAT AND PIG

M. H. Weeks, L. A. George, W. D. Cakley
L. K. Bustad, and R. C. Thompson

The absorption of plutonium from the gastro-intestinal tract of 3 pigs averaged 0.0022 per cent, with 95 per cent confidence limits of ± 0.0013. This figure does not differ significantly from the results of more extensive experiments on rats.

Previous chronic feeding experiments done in this laboratory have established an average value of 0.0028 per cent for absorption of plutonium from the gastro-intestinal tract of rats (1, 2). In extending these studies to larger animals, chronic feeding was considered impractical. Large single doses were therefore fed to both pigs and rats. The results, combined with earlier data, afford a comparison between acutely dosed pigs and rats and between acute and chronically dosed rats. A more detailed account of these experiments will be found in HW-33744 (3).

METHODS

Three pigs (female, Chester-White x Danish Landrace hybrids*) and 10 rats (female, Sprague-Dawley) were given single stomach tube feedings of a Pu^{239} nitrate solution, pH 2, containing 0.45 mg Pu (rats) and 9.0 mg Pu (pigs). The animals were sacrificed nine days later and representative tissues, as well as total skeleton and soft tissues, analyzed for plutonium content. Excreta from the rats were collected and analyzed.

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* These pigs were obtained from the Department of Animal Husbandry, State College of Washington, Pullman, Washington.

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RESULTS AND DISCUSSION

A summary of the results on the pig (single feeding) and the rat (single feeding and chronic feeding) is given in Figure 1. Differences in total absorption between the two species are clearly not significant. The tendency toward an increased soft tissue deposition in the singly fed animals may be reasonably explained in terms of a gradual shift of plutonium, with time, from the soft tissues to bone.

Analyses on excreta from the singly fed rats indicated that less than one per cent of the plutonium fed remained in the intestine after 48 hours. Less than 20 per cent of the total plutonium absorbed was excreted in the urine.

The demonstrated close similarity in extent of gastro-intestinal absorption of plutonium in the rat and pig is presumptive evidence for a similar behavior in the human. The presently recommended maximum permissible concentration of plutonium in drinking water (1.5×10^{-6} $\mu c/ml$) (4) is based on an assumed absorption of 0.1 per cent. In view of the data here presented, this figure would seen to be unnecessarily conservative by a factor of at least ten.

ACKNOWLEDGMENTS

We wish to acknowledge the technical assistance of Joan O. Hess and Elizabeth C. Desposato.

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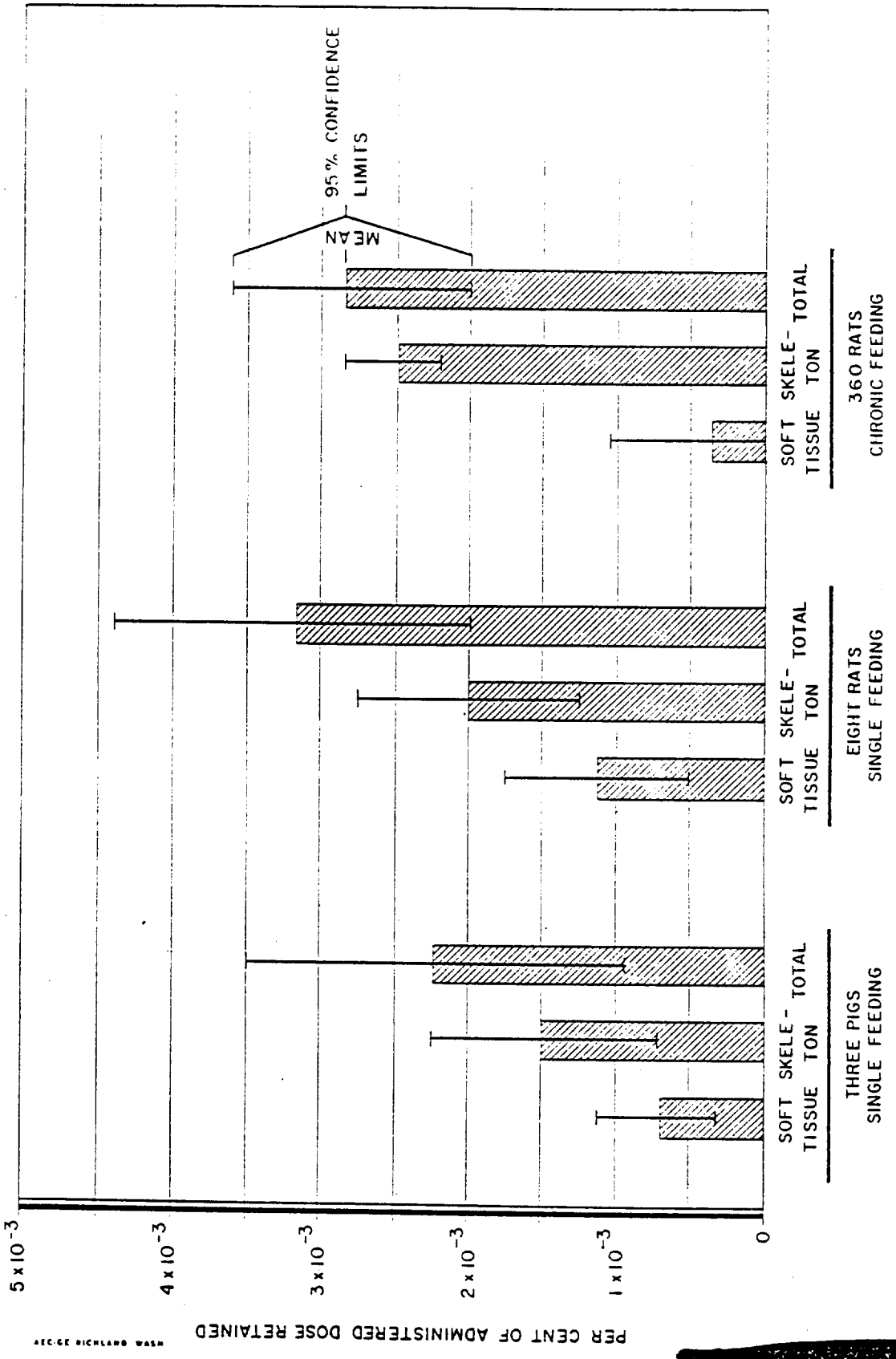

FIGURE I

COMPARISON OF GASTROINTESTINAL ABSORPTION OF PLUTONIUM IN THE RAT AND PIG

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ELECTROPHORETIC STUDIES OF THE DISTRIBUTION OF PLUTONIUM AND RUTHENIUM IN THE BLOOD OF RATS

R. F. Palmer

Preliminary observations of combination of plutonium and ruthenium with plasma proteins are described.

In support of programs involving the metabolism of plutonium and ruthenium, electrophoretic studies of the distribution of these elements in the blood of rats were initiated.

METHODS

A Spinco Model R paper electrophoresis apparatus, employing the free hanging vertical strip method of Durrum (1), was used to fractionate blood serum aliquots obtained from rats following administration of either Ru^{106} or Pu^{239}. Electrophoretic separations were performed at a pH of 8.6 in a barbiturate - HCl buffer having an ionic strength of 0.05. A constant current of 5.0 ma resulted in migration of the albumin fraction 12 to 14 cm from the origin after 16 to 18 hours. Strips were stained with bromphenol blue and 0.25 cm sections counted for Ru^{106} or Pu^{239} content.

RESULTS

Both plutonium and ruthenium in the blood were found to exist predominantly in association with plasma proteins. The distribution of Ru^{106} (III) among the protein fractions was the same 3 minutes and 60 minutes after intravenous injection. Approximately 30 per cent of the activity was associated with the albumin fraction, 25 per cent with the α_2-globulin, 10 per cent with the α_1-globulin, and 15 per cent with the

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β-globulin. Sixty minutes after intraperitoneal injection of Ru^{106} (III) a larger fraction was found associated with the α_1-globulin, and smaller fractions with the α_2-globulin and albumin.

Preliminary observations on blood from a rat injected intravenously with Pu(IV) citrate indicated that more than 50 per cent of the Pu was associated with the β-globulin fraction, in agreement with a previous report of Müntz and Barron (2).

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GASTRO-INTESTINAL ABSORPTION OF RUTHENIUM IN THE RAT (PRELIMINARY STUDIES)

R. C. Thompson and O. L. Hollis

Approximately one per cent of ruthenium administered as a pH 2 chloride solution via stomach tube was absorbed and retained in the animal after 24 hours. Percentage absorption decreased with lowered acidity of the administered solution and appeared to increase with addition of large amounts of inactive ruthenium carrier.

In our previous Annual Report, Hanson and Browning reported an approximately 3 per cent absorption of ruthenium administered intragastrically to chickens (1). This result was so much higher than the <0.05 per cent absorption in rats previously reported (2, 3) that experiments were instituted to re-evaluate ruthenium absorption in the rat. The experiments reported here consist of preliminary studies of the effect of pH of solution administered, and the effect of total ruthenium concentration on gastro-intestinal absorption. More extensive studies of ruthenium metabolism will be found in the following papers of this Annual Report.

METHODS

The Ru^{106} employed in these experiments was obtained from Oak Ridge as "ruthenium chlorides" in HCl solution. Dilutions of this solution were adjusted to the desired pH with NaOH and allowed to stand for 24 hours before administration. Where addition of carrier ruthenium was required, the mixture of tracer and carrier was oxidized with $HClO_4$, distilled as RuO_4 into HCl and reduced with SO_2 to insure chemical uniformity of the ruthenium solution.

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Animals employed were female Holtzman rats fed ad libitum on a commercial pelleted diet. One milliliter of ruthenium solution was administered by stomach tube and the animals sacrificed 24 hours later. Tissue samples were mascerated on stainless steel plates, air dried, and counted. Self-absorption was negligible.

RESULTS AND DISCUSSION

Table 1 shows the concentration and distribution of ruthenium in various tissues following the intra-gastric administration of solutions of pH 2.3, 3.8, and 6.2. Although only two animals were fed in each group, there is an obvious trend toward decreased absorption at lower acidities, probably due to decreased solubility. Additional experiments with pH 2 solutions confirmed the absorption and retention after 24 hours of approximately one per cent of the ruthenium fed.

Increasing carrier ruthenium to 2 mg/ml, a factor of 1,000 greater than the ruthenium concentration in the Oak Ridge solution, increased the per cent absorbed by a factor of approximately 4. The oxidation, distillation, and reduction treatment applied to the ruthenium in the "effect of carrier" experiment appeared to affect significantly the absorbability of the ruthenium. Without addition of carrier, this treatment reduced absorption threefold, as compared with the untreated Oak Ridge solution. It is therefore not clear just what effects should be attributed to the increased ruthenium concentration and what effects may be due to possible changes in valence state occasioned by the chemical treatment. Additional experiments are in progress to resolve this problem. In any case, the gross uptake is substantially greater than that reported in the earlier literature.

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TABLE 1

Effect of pH of Solution Fed on the Absorption of Ruthenium from the Gastro-Intestinal Tract of Rats
(Average Results from 2 Rats Per Group)

Tissue	Per Cent of Administered Dose Per Gram		
	pH 2.3	pH 3.8	pH 6.2
Kidney	0.16	0.10	0.029
Liver	0.034	0.023	0.0046
Spleen	0.022	0.0082	0.0024
Blood	0.011	0.0079	0.0024
Lungs	0.016	0.0059	0.0021
Heart	0.011	0.0040	0.0012
Bone	0.0090	0.0028	0.0010
Muscle	0.0048	0.0018	0.0005
Fat	0.0057	0.0016	0.0008
Brain	0.0016	0.0004	0.0002
Per Cent Absorbed in Total Animal	1.4	0.75	0.22

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The relative distribution of absorbed ruthenium among the tissues studied was not greatly affected by the variables introduced in these experiments. At high inert ruthenium concentrations there is an indication that the ruthenium concentration in the liver may be increased relative to that in the kidney. In all experiments the kidney showed the highest ruthenium concentration, 24 hours after administration.

ACKNOWLEDGMENTS

We wish to acknowledge the technical assistance of Joan O. Hess and Margaret B. Lawson in this work.

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ABSORPTION, DISTRIBUTION, AND RETENTION OF RUTHENIUM IN RATS FOLLOWING CHRONIC ORAL ADMINISTRATION

M. H. Weeks and R. C. Thompson

About 3 per cent of ruthenium chronically administered to rats is absorbed from the G. I. tract. The ratio of urinary to fecal excretion of absorbed ruthenium is approximately 4 to 1. Kidney accumulates the highest concentration of ruthenium over a 71-day feeding period. Most tissues attain an equilibrium concentration of ruthenium after about 30 days' feeding, but bone and spleen are notable exceptions to this rule.

Several experiments were initiated to determine the effect of chronic ruthenium feeding on the absorption and accumulation of ruthenium in various organs and tissues of the rat. None of these experiments are completed, and other experiments are planned to supplement those already in progress. Any conclusions drawn from the preliminary results here described must therefore be considered as quite tentative.

METHODS

The separate experiments performed all involved the administration of Ru^{106} (Oak Ridge "carrier-free chlorides"), diluted and adjusted to pH 2. Separate groups of animals were given the ruthenium solution by stomach tube or by inclusion in their drinking water. Details of the feeding and sacrifice schedules of the separate experiments are given in Table 1. Daily urine and feces collections were made from 5 to 8 animals in the 2 μc intubation experiment. All tissue samples were mascerated, air-dried, and counted directly with an end-window geiger counter, with the exception of feces samples which were partially digested with nitric acid. Counting losses due to self-absorption and losses during digestion of the feces samples were negligible.

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TABLE 1

Design of Ruthenium Chronic Feeding Experiments

Designation of Experiment	Dose Administered	Frequency of Dosage	Avg. Daily Dose	Duration of Feedings	No. of Animals		Disposition of Animals
					M	F	
"2 μc intubation"	1.8 μc	Daily, 5 days per week	1.3 μc	71 days	86		Groups of 4 sacrificed at 1, 10, 17, 24, 31, and 52 days; 24 hours after last previous stomach tube feeding. Following cessation of feedings, groups of 4 sacrificed at 1, 2, 5, 8, 12, and 16 days. Remainder of animals to be sacrificed at later intervals.
"0.1 μc intubation"	0.12 μc	Daily, 5 days per week	.085 μc	71 days	5	5	Sacrificed 24 hours after last feeding.
"0.1 μc drinking water"	0.1 μc/ml water ad lib.	Continuous	4.3 μc	71 days -(males) >71 days (females)	10	10	Ten males sacrificed at conclusion of 71-day drinking water regimen. Females remain on regimen to be sacrificed at a later date.

RESULTS AND DISCUSSION

Excretion curves from the 2 μc intubation experiment, covering the latter portion of the feeding period and the first 30 days post feeding, are shown in Figure 1. Points plotted during the feeding period are averages for a week, but include only the collections made during the 24 hours following a feeding. On week ends, when ruthenium was not administered, excretion dropped markedly.

An average of 2.4 per cent of the total ruthenium excreted during the later portion of the feeding period appears in the urine. This figure, plus any ruthenium excreted from the body via secretion into the G. I. tract, should be a measure of the ruthenium absorbed from the G. I. tract, since the ruthenium body burden is essentially constant. From the ratio of urinary to fecal excretion of ruthenium during the period 10-30 days post feeding, it can be concluded that excretion of absorbed ruthenium via the G. I. tract amounts to about one-fourth of the urinary excretion. This ratio might not hold during the feeding period, but as a first approximation, adding 25 per cent to the urinary excretion of 2.4 per cent gives a figure of 3 per cent for total absorption during chronic feeding. This compares with reported figures of 3 per cent absorbed and retained for 24 hours in chickens (1), and <0.05 per cent for rats (2, 3).

Figure 2 shows build-up and retention curves for seven organs and tissues obtained from the 2 μc intubation experiment. Build-up curves for most tissues approach equilibrium levels within about 30 days. Bone and spleen are notable exceptions, showing a steady increase in ruthenium concentration during the 30-71 day interval. Kidney accumulates the highest ruthenium concentration and, as previously assumed (3), would appear to be the critical organ. However, bone and spleen cannot be ruled out as contenders for critical organ status in view of their continuing build-up of ruthenium throughout the feeding period, and their more tenacious retention of ruthenium following feeding.

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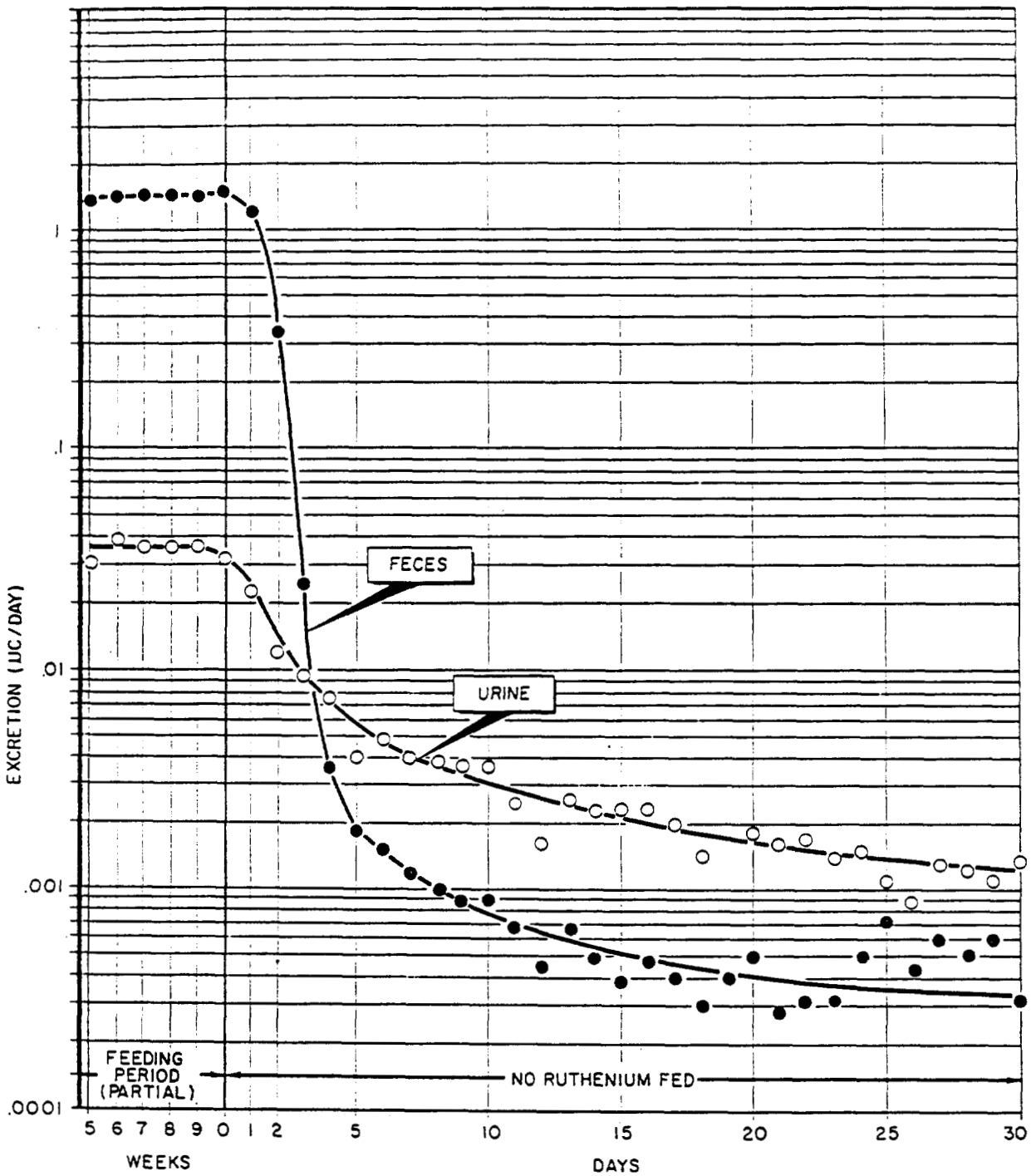

FIGURE 1
EXCRETION OF Ru^{106} DURING AND FOLLOWING CHRONIC FEEDING

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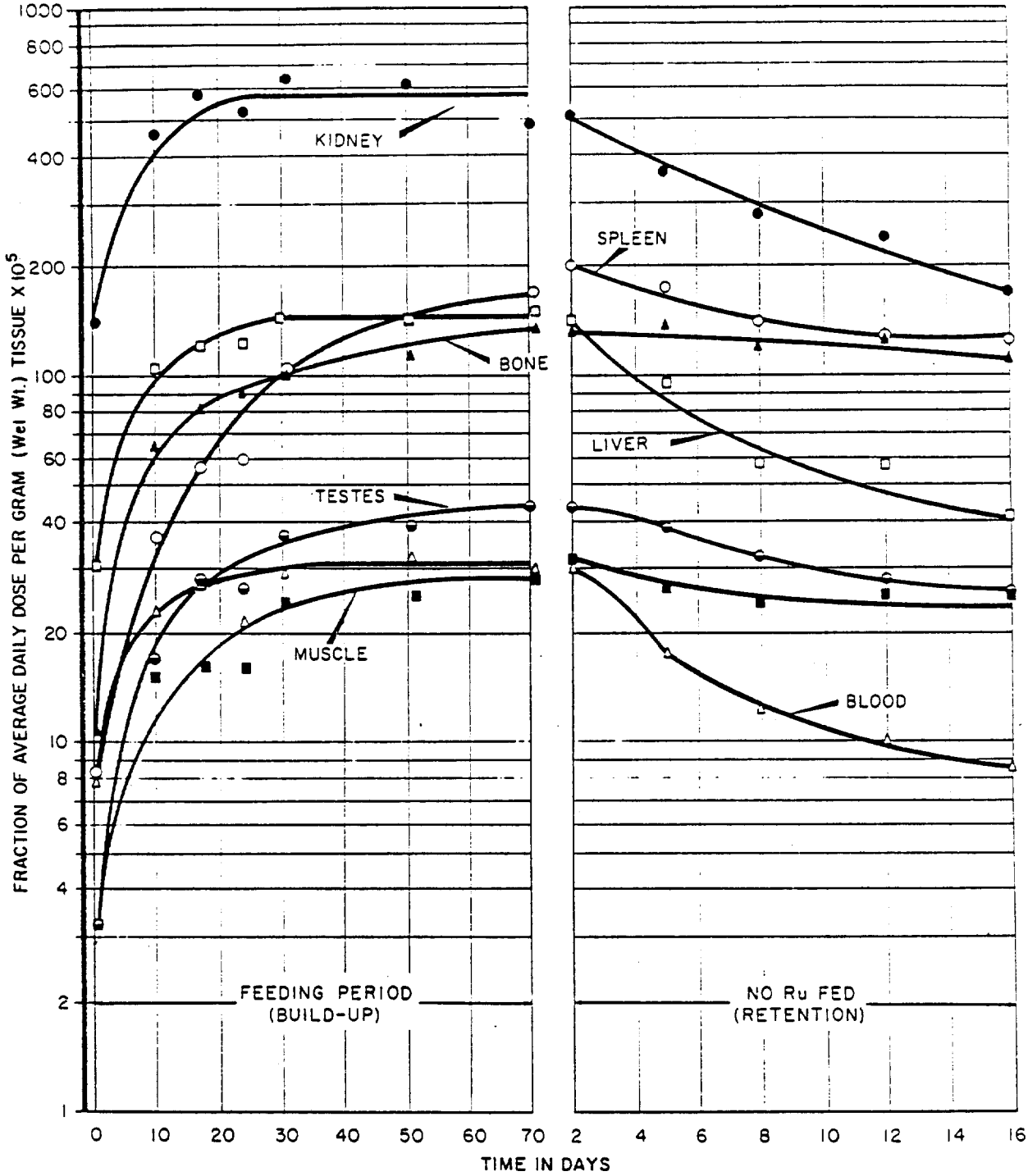

FIGURE 2

CONCENTRATION OF Ru^{106} IN TISSUES DURING AND FOLLOWING CHRONIC FEEDING

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The data on ruthenium retention thus far obtained are too limited for drawing conclusions with regard to biological half-life. With the possible exception of bone, all tissues seem to contain components with different half-lives.

Table 2 compares results from the three chronic feeding experiments. Data are calculated on a total organ basis to minimize effects of weight differences. Agreement between the experiments is reasonable, considering the small numbers of animals involved.

If one assumes that 2.5 per cent of the chronic daily intake of ruthenium will be found in the kidneys, and applies this figure to the calculation of an MPC for drinking water for man, a value of 0.003 μc/ml is obtained. This may be contrasted with the present recommended value of 0.1 μc/ml, which is based on an assumed absorption from the gastrointestional tract of 0.05 per cent (3).

ACKNOWLEDGMENTS

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

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TABLE 2

Ruthenium Organ Burdens Following Chronic Feeding - Comparison of Experiments

Experiment[1]	Fraction of Avg Daily Dose per Organ			
	Kidneys	Liver	Testes	Bone[2]
"2 μc intubation" Avg of 7 males	0.014	0.014	0.0026	0.035
"0.1 μc intubation" Avg of 5 males	0.024	0.024	0.0035	0.035
Avg of 4 females	0.030	0.027		0.046
"0.1 μc drinking water" Avg of 8 males	0.026	0.026	0.0032	0.047

[1] See Table 1 for details of experiment.

[2] Extrapolated from analyses on femurs, assuming bone to constitute 6.25 per cent of body weight.

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DISTRIBUTION AND RETENTION OF RUTHENIUM IN RATS FOLLOWING INTRAPERITONEAL ADMINISTRATION

R. C. Thompson and O. L. Hollis

Early results are reported from an experiment to study the long-term retention of ruthenium in the rat. Kidney shows the highest initial retention; bone, the slowest turnover.

Distribution and retention of ruthenium in the rat following intraperitoneal administration was studied by Scott, et al (1) in an experiment involving 12 animals, sacrificed in four groups, at intervals up to 64 days. Results have also been reported on the distribution and retention of ruthenium in rats following inhalation (2). The present experiment was designed to obtain more extensive data on ruthenium retention which could be compared with similar data from chronic feeding studies (3), and from which the biological half-lives in various rat tissues might be deduced.

METHODS

Thirty-nine Sprague-Dawley female rats, averaging 250 g, were injected intraperitoneally with 7.7 μc of Ru^{106} (Oak Ridge "carrier-free chlorides"). Groups of four rats each are to be sacrificed at intervals up to 256 days post injection. Samples of blood, kidneys, liver, spleen, lungs, heart, brain, ovaries, fat, muscle, bone (femurs), stomach (contents removed), large and small intestines (contents removed), were mascerated on stainless steel plates, air dried, and counted directly with an end-window Geiger counter. Self-absorption was negligible. Samples of belly muscle adjacent to the site of injection, and the residual carcass, minus pelt, feet, head and tail, were digested with nitric acid and aliquots of the solution plated for counting. Ruthenium losses during digestion are negligible if care is taken not to allow evaporation to dryness.

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RESULTS AND DISCUSSION

Results from the first six groups of animals sacrificed are shown in Table 1. An additional three groups remain to be sacrificed at later intervals. Figure 1 shows the retention curves for some of the more interesting organs and tissues. None of the data have been corrected for radioactive decay, which would be quite negligible over the period thus far represented in the study (half-life of Ru^{106} = 365 days).

Any attempt at calculation of biological half-lives or close comparison with similar data from other sources must await the completion of the experiment with data beyond 31 days. At present it can be said that the results are qualitatively similar to those reported by Scott, et al (1), and to those obtained from chronic feeding studies (3). The general level of tissue incorporation appears to be 2-5 times higher than reported by Scott, et al, and the turnover rates somewhat slower.

ACKNOWLEDGMENTS

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

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TABLE 1

Concentration of Ruthenium in Tissues Following Intraperitoneal Injection of Ruthenium Chloride (Expressed as per cent of dose per gram of tissue)

Group No. Time Killed, Days Avg Rat Wt g	1 1 248	2 2 240	3 4 246	4 8 255	5 16 255	6 31 259
Kidney	2.2	2.9	3.1	2.9	2.1	1.3
Liver	1.8	1.9	1.6	1.6	0.88	0.34
Spleen	2.2	1.8	2.6	2.8	1.6	1.0
Lungs	0.77	0.52	0.50	0.35	0.20	0.11
Heart	0.38	0.29	0.22	0.16	0.14	0.079
Brain	0.034	0.022	0.015	0.0069	0.0035	0.0020
Ovaries	1.2	1.1	1.4	1.3	0.88	0.43
Fat					0.27	0.15
Muscle	0.10	0.11	0.086	0.076	0.050	0.025
Bone	0.31	0.29	0.32	0.24	0.27	0.18
Stomach	0.41	0.33	0.32	0.34	0.20	0.099
Large Intestine	0.77	1.1	0.79	0.68	0.35	0.14
Small Intestine	0.83	0.67	0.62	0.51	0.30	0.14
Blood	0.94	0.64	0.26	0.086	0.016	0.0035
Belly Muscle[1]	0.32	0.30	0.34	0.23	0.17	0.077
Residual Carcass[2]	0.17	0.15	0.14	0.11	0.078	0.077

[1] A sample of muscle from the anterior abdominal wall at site of injection.

[2] Residual carcass excludes pelt, feet, head and tail.

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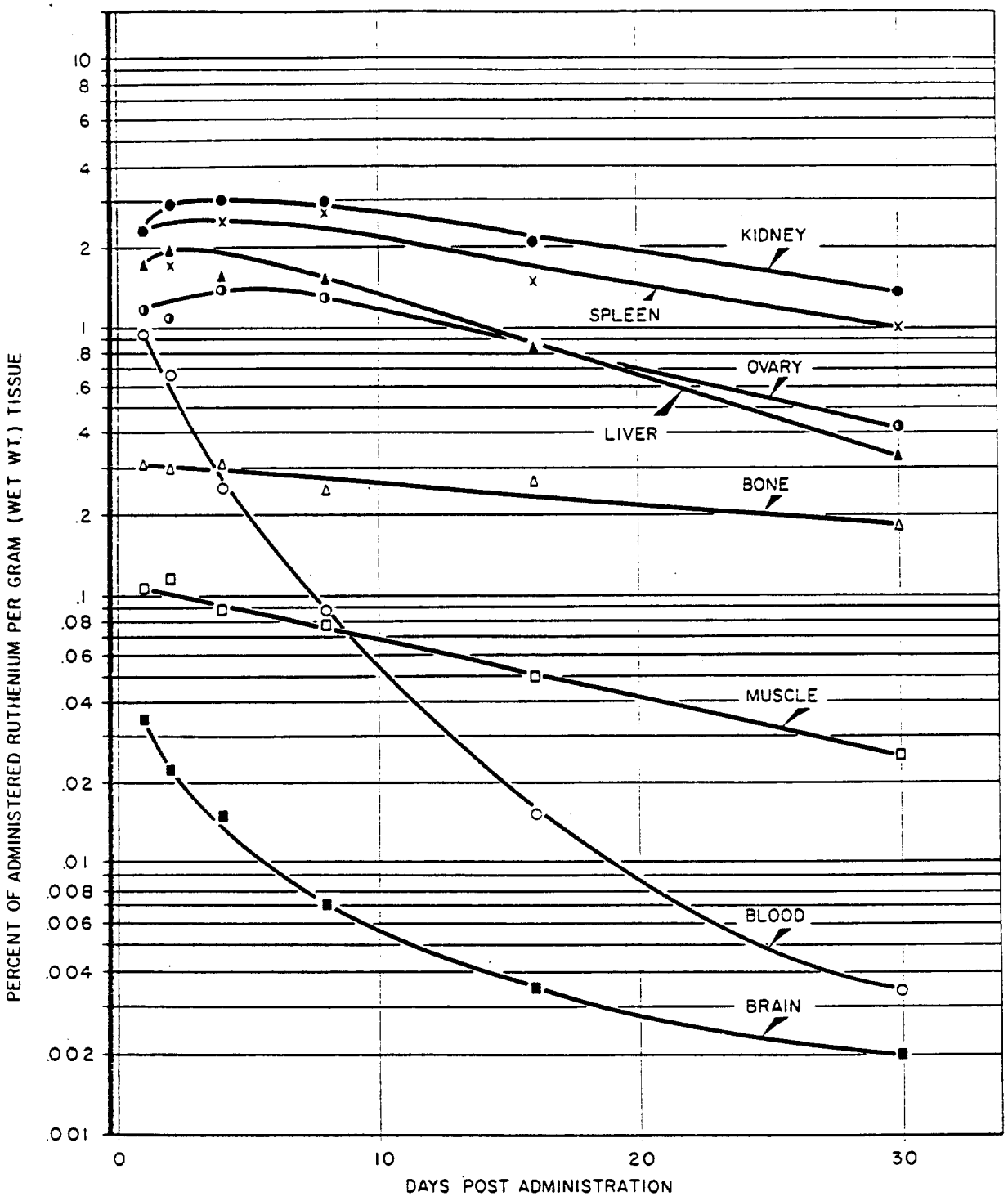

FIGURE I
RETENTION OF RUTHENIUM IN THE RAT
FOLLOWING INTRAPERITONEAL ADMINISTRATION

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

R. C. Thompson and O. L. Hollis

The tissue distribution of tritium in rats was determined as a function of time following acute exposure to tritium gas. Results are compared with similar data obtained following administration of tritium oxide. While certain differences are noted in the pattern of tritium incorporation and retention following the two types of exposure, these differences are not significant in the evaluation of the over-all hazard.

Preliminary studies of the incorporation of tritium into tissue compounds following exposure of rats to tritium gas were previously reported (1). The possibility of direct incorporation of elemental tritium into tissue compounds was suggested by the high initial bound tritium concentrations in certain tissues. From the standpoint of hazard evaluation this question is of some importance, since it may often be difficult, if not impossible, to determine whether accidental contamination of humans occurs as a result of exposure to the gas or to the oxide. The only convenient method of evaluating the extent of human exposure is by measuring the tritium oxide content of urine, which is assumed to bear a constant relationship to body tritium content regardless of the manner in which the exposure occurred. The experiment here described was designed to evaluate initial incorporation of tritium following exposure to the gas, and also retention of this bound tritium over a period of 80 days following exposure.

METHODS

Twenty-four mature, Sprague-Dawley, female rats, averaging 215 g, were exposed in groups of 6, for 4 hours, to tritium gas atmospheres. The apparatus and procedures involved were described in the

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previous Annual Report (1). Tritium concentrations in the exposure chamber averaged 500, 205, 300, and 280 μc/cc during the four separate exposures. The exposed animals were divided into six groups of four which were sacrificed at 1 hour, 3, 7, 20, 40, and 80 days following exposure. Each sacrifice group was composed of one animal from each of the 4 exposure groups, and samples from a given sacrifice group were pooled. The average tritium exposure for each sacrifice group was therefore the same despite the variation among the exposure groups.

Various organ and tissue samples were removed at sacrifice. The residual carcass was thoroughly ground and two 10 g aliquots taken for analysis. The procedures employed for removal of tissue water, combustion of dry samples and analysis for tritium have been previously described (2, 3). Samples were not equilibrated with water to remove freely exchangeable tritium except in the case of one of the duplicate residual carcass samples.

RESULTS AND DISCUSSION

Concentrations of bound tritium were determined for various organs and tissues at various time intervals following total body exposure to tritium gas. These concentrations resulted from a 4-hour exposure to an average tritium concentration in the atmosphere of 320 μc/cc.

It was the purpose of this experiment to compare these bound tritium values following exposure to tritium gas with those obtained in a previous experiment in which tritium oxide was administered by intraperitoneal injection. Results of the tritium oxide study have been previously reported (4). In the present gas exposure experiment the concentration of tritium oxide in body water immediately following exposure was 43 μc/ml. In the earlier oxide exposure experiment the level of tritium oxide in body water one day following injection was

90 μc/ml. To compare the results of these two experiments it is necessary to take this difference in exposure levels into account. The tissue-bound tritium concentrations from the two experiments were therefore divided by the initial body water tritum concentrations. A value of 100 μc/ml was assumed for the initial body water level in the tritium oxide experiment to allow for loss of tritium oxide during the first 24 hours following exposure. The normalized values of bound tritium concentration thus obtained may be expressed as a fraction:

$$\frac{[\text{Bound tritium}]_{\text{HT exp.}}}{[\text{Initial body water HTO}]_{\text{HT exp.}}} \Big/ \frac{[\text{Bound tritium}]_{\text{HTO exp.}}}{[\text{Initial body water HTO}]_{\text{HTO exp.}}}$$

The value of this fraction should be unity if incorporation of tritium following exposure to tritium gas occurs only as a consequence of the metabolism of tritium ions from the body water. A value significantly greater than unity indicates the operation of some additional mechanism of incorporation peculiar to the conditions of exposure to tritium gas.

Table 1 lists such fractions as described above for four time intervals following exposure. The time intervals are not exactly the same in the two experiments, but are sufficiently close to yield meaningful comparisons. It will be seen that for the majority of tissues these fractions are within a factor of two of unity. Differences of this magnitude can hardly be considered significant in view of the problems inherent in the comparison of biological experiments performed two years apart. Certain obviously significant differences are apparent, however. Initial tritium incorporation in fat appears to be much lower than would be predicted from the body water tritium content, while initial tritium incorporation in muscle is much higher. To a lesser degree, incorporation of tritium in bone and pelt is also high and the total carcass also reflects a higher than predicted bound tritium content.

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TABLE 1

Comparison of Tritium Incorporation
Following Exposure to Tritium Gas and Tritium Oxide

Time of Sacrifice	HT Exposure	1 Hour	7 Days	20 Days	80 Days
	HTO Exposure	1 Day	6 Days	20 Days	83 Days

Tissue-Bound Tritium Following Exposure to Gas, Expressed as Fraction of That Predicted from Initial Tritium Oxide Content of Body Water*

Heart	1.5	1.6	1.6	1.6
Lungs	1.0	1.0	1.6	--
Liver	1.1	1.1	1.7	0.9
Kidneys	1.4	1.3	1.8	0.7
Stomach	0.9	1.[illegible]	2.0	1.4
Small Intestine	0.7	1.0	1.8	[illegible]
Large Intestine	1.1	1.0	1.5	1.2
Brain	1.4	1.2	1.7	1.6
Blood	1.4	0.9	1.9	0.6
Fat	0.1	0.4	1.0	2.7
Muscle	21	0.9	1.6	1.4
Bone	3.3	0.9	0.5	0.3
Pelt	4.1	1.6	1.3	--
Carcass (total)	2.2	0.9	1.3	1.2

$$* \quad \frac{[\text{Bound tritium}]_{\text{HT exp.}}}{[\text{Initial body water HTO}]_{\text{HT exp.}}} \Big/ \frac{[\text{Bound tritium}]_{\text{HTO exp.}}}{[\text{Initial body water HTO}]_{\text{HTO exp.}}}$$

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Despite these marked differences in early incorporation in certain tissues, the general similarity in the incorporation processes following the two types of exposure is indicated by the very close agreement of the total carcass curves (Figure 1) after 6 days. These curves are normalized to represent the same levels of tritium oxide in body water following exposure. The early differences between the two exposure methods are interesting from the point of view of possible mechanisms involved. The differences are of little hazard significance, however, since they are of a transient nature. Any significant hazard from bound tritium lies in its potential retention for long time periods (5).

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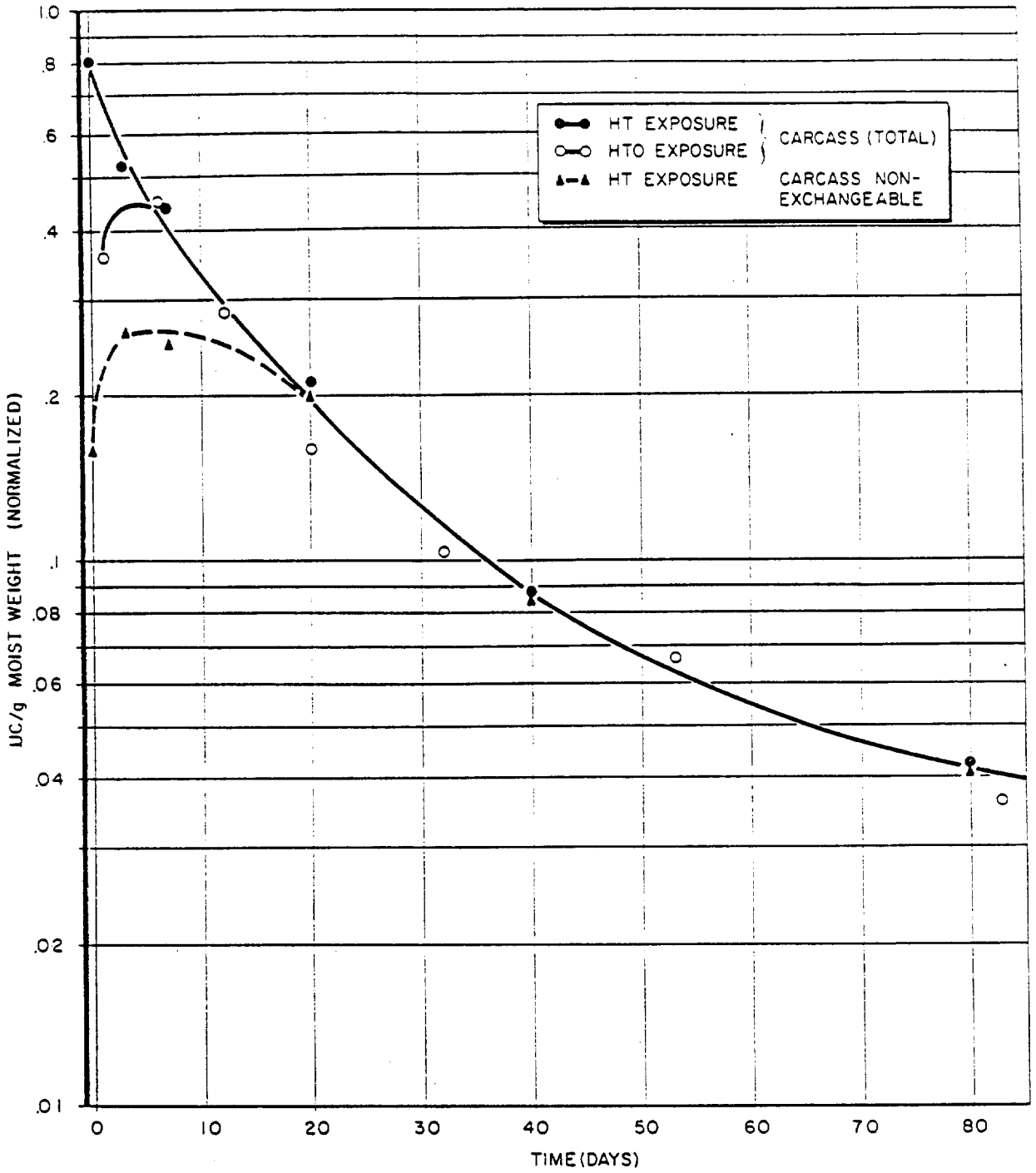

FIGURE I
RETENTION OF BOUND TRITIUM FOLLOWING EXPOSURE TO TRITIUM GAS AND TRITIUM OXIDE

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

R. C. Thompson and J. E. Ballou

Rats were exposed to a constant tritium oxide environment from conception to age six months. Retention of organically-bound tritium in these animals was followed over a period of 300 days following the exposure. Components of slow turnover rate (half-lives from 70-300 days) constitute a significant fraction of most tissues - from 40-70 per cent of muscle, brain, pelt, fat and bone.

By exposing animals to a constant tritium oxide environment from conception, one should approach the condition in which the oranically-bound hydrogen of these animals is uniformly labeled with tritium. In such animals the organically-bound-tritium contents of different components of the animal should be approximately proportional to the mass of the components. Such a uniformly labeled animal would offer obvious advantages in the study of metabolic turnover rates.

The present experiment was designed to produce such animals and utilize them in several ways. Preliminary results were described in the previous Annual Report, including data on the tritium levels attained at the end of the exposure period and conclusions drawn from these data with respect to the increased radiation exposure hazard attributable to organically-bound tritium (1). In this report we wish to consider the results obtained on retention of organically-bound tritium in organs and tissues of these animals over long time intervals following their exposure.

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METHODS

Twelve mature, Sprague-Dawley, female rats were maintained on a constant tritium oxide-labeled drinking water regimen (5 μc/ml) for four months. During this period the animals were bred, and 38 young were weaned to the same tritium oxide drinking water regimen, which was continued to age six months. The original females were sacrificed in groups of two, at intervals of 0, 10, 30, 93, 184 and 360 days after removal from the tritium oxide regimen. The offspring were sacrificed in groups of 3 at intervals of 0, 5, 14, 35, 70, 119, 200 and 300 days after removal from the tritium oxide regimen. Organically-bound tritium was determined in various organs and tissues from these animals. Various lipid and protein fractions are also being separated and analyzed - this phase of the problem is incomplete and will not be reported on at this time. A more detailed account of experimental procedure and methods employed was given in the previous Annual Report (1).

RESULTS AND DISCUSSION

The bound tritium contents of the various organs and tissues were normalized to constant weight. Retention curves were plotted on semi-log paper and resolved into exponential components. The half-lives of these components, and the fraction of total bound tritium in each organ or tissue which is represented by each component, is shown graphically in Figure 1. These results are shown only for the second generation animals which were exposed to the constant tritium oxide environment from conception. Similar results were obtained from the first generation animals exposed for four months.

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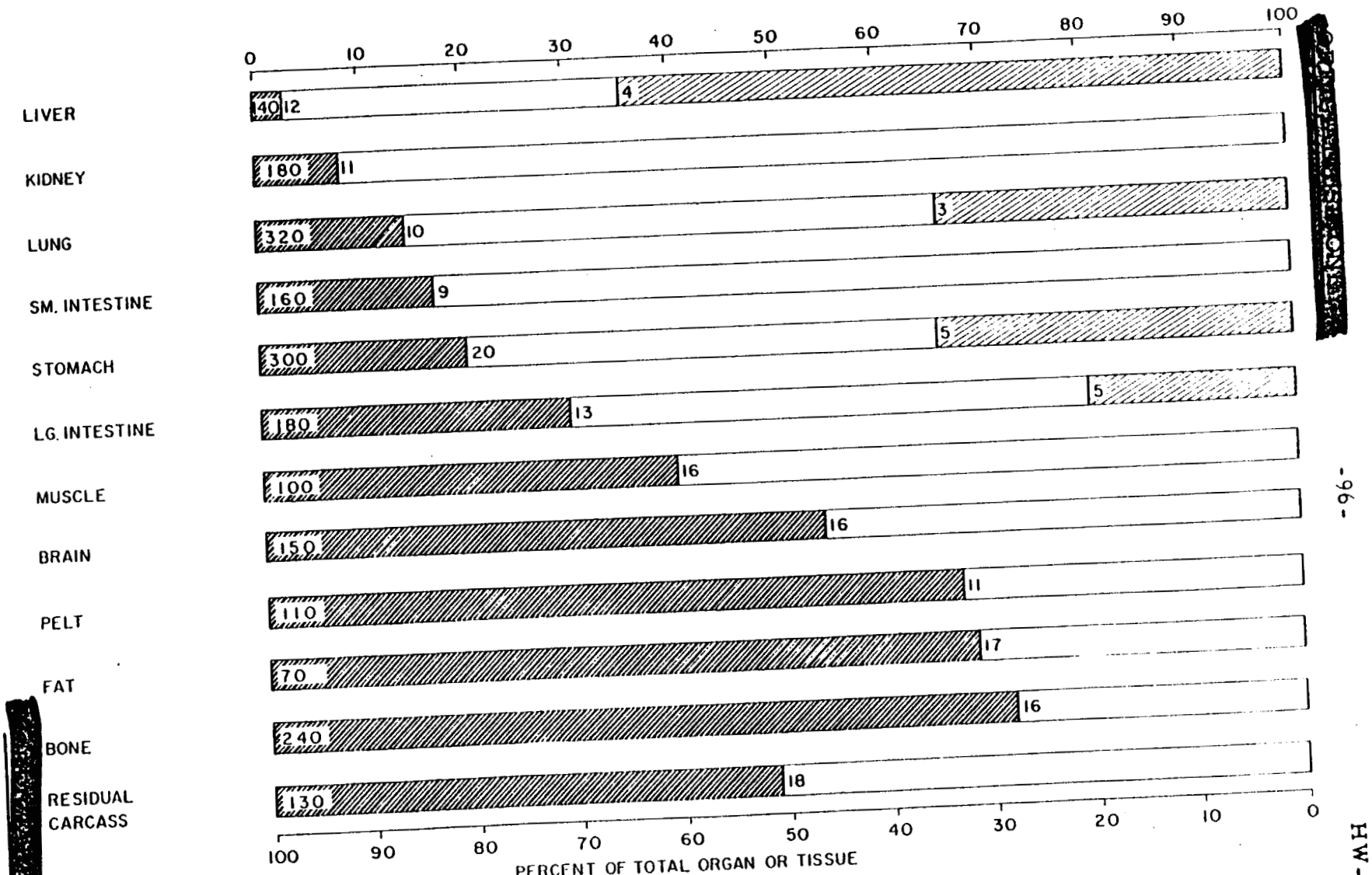

FIGURE I

MAGNITUDE AND BIOLOGICAL HALF-LIFE OF TRITIUM BINDING COMPONENTS IN THE RAT

(LENGTH OF BAR IS PROPORTIONAL TO SIZE OF COMPONENT; NUMBER IN EACH BAR IS THE BIOLOGICAL HALF-LIFE IN DAYS)

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There was clear evidence in all retention curves for a long-lived component of half-life from 70-300 days. A component of intermediate half-life, from 9-20 days, was also present in all tissues. A short-lived component of 3-5 days was resolvable in several cases. Such short-lived components were undoubtedly present in all tissues, but the preponderance of the long-lived and intermediate components and the paucity of points during the early portion of the retention curves made it impossible to accurately resolve such short-lived components from most of the curves.

It is felt that these results conclusively demonstrate the existence in the rat of widely distributed, metabolically inert constituents which are not significantly involved in any dynamic state of destruction and resynthesis. These inert constituents are quantitatively of minor significance in organs of high metabolic activity, such as liver and kidney, but assume quantitative predominance in such tissues as muscle, fat and bone. About one-half of the total animal is constituted of such metabolically undynamic material.

ACKNOWLEDGMENTS

We wish to acknowledge the technical assistance of Margaret B. Lawson.

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TRITIUM OXIDE ABSORPTION AND RETENTION IN THE BODY WATER OF SOME AQUATIC ORGANISMS

R. F. Foster

Salmon fry, mayfly nymphs, and snails immersed in water containing tritium oxide incorporated the tritium into their body water at a rate corresponding to a half-time of about 15 minutes. In an uncontaminated environment the half-time for elimination varied from about 10 minutes for the snails to about 24 minutes for the small fish.

Considerable attention has been given to tritium and tritium oxide metabolism in mammals (1, 2). Rates of absorption of the two gases through skin and lungs of mammals have been defined as well as distribution and turnover for several body constituents. However, no similar work has previously been done on aquatic animals. To determine tritium oxide absorption and retention in animals representing widely different body forms, studies were made on snails, insect nymphs and young fish.

METHODS

Chinook salmon fry (Oncorhynchus tshawytscha), weighing approximately one gram each, were immersed in water with a tritium oxide concentration of 0.29 μc/ml. Two or three of the fry were sampled at intervals ranging from one minute to eight hours, and the concentration of tritium in their body water determined. In a second test, the fry were first exposed to water containing 0.33 μc T/ml for 24 hours, then placed in flowing, tritium-free water for intervals ranging from one minute to eight hours.

Similar exposures to tritium oxide solutions and to tritium-free water were made with mayfly nymphs (Callibaetis sp.) and with snails (Radix japonica). Since the mayfly nymphs weighed only about 10 mg each,

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50 individuals were used for each sample. The snails weighed approximately one gram each. Their shells were removed before analyzing for tritium. All tests were made at approximately 20°C in Columbia River water that contained 95 ppm total solids.

RESULTS AND DISCUSSION

If the body water of the aquatic animals exchanges freely with the surrounding water, then the rate of increase in tritium oxide concentration in the organism should be proportional to the difference between the HTO concentration in the organism and the environment. For a simple system this can be expressed as:

$$\frac{dC_b}{dt} = k\,(C_e - C_b)$$

C_b = concentration of HTO in organisms
C_e = concentration of HTO in environment
k = specific reaction rate constant
t = time

Integrated and rewritten, this expression becomes:

$$\log \frac{C_e}{C_e - C_b} = \frac{k}{2.3}\,t$$

When the fraction $\frac{C_e}{C_e - C_b}$ is plotted on a semilog scale against time, a straight line should result if the initial assumption is correct. The resulting curves for the three organisms tested are shown in Figure 1. The curves are remarkably similar and, for the first half-hour, suggest that these animals replace half of their body water about every fifteen minutes. As the tritium concentration of the body water approaches that of the surrounding medium, the rates fall significantly below the theoretical straight line. Even after several hours' exposure the tritium oxide

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concentration within the animals amounted to only about 95 per cent of that of the environment. This might indicate the existence of a small body-water pool with an appreciably slower turn-over rate.

The rate of elimination of tritium from the body is plotted on semi-log coordinates in Figure 2. Straight lines have been fitted by inspection to the data for the three organisms. Half-time elimination rates appeared to be about 10 minutes for snails, 20 minutes for mayflies, and 24 minutes for salmon fry. While these values are somewhat at variance with the 15 minute half-time suggested by the uptake tests, the rates are not incompatible. The rate-of-elimination curves may best represent the turnover rate for the bulk of the body water since the tritium concentration of any "remote" pools may not have become equilibrated and thus would not significantly distort the curve presumed to represent only the freely exchangeable body water. The comparatively good fit of straight lines to the snail and salmon fry data with the semi-log coordinates of Figure 2 strengthens this hypothesis. The faster turnover of body water in the snail possibly reflects a more permeable integument.

ACKNOWLEDGMENTS

The technical assistance of R. L. Gruver and H. J. Knauss is gratefully acknowledged.

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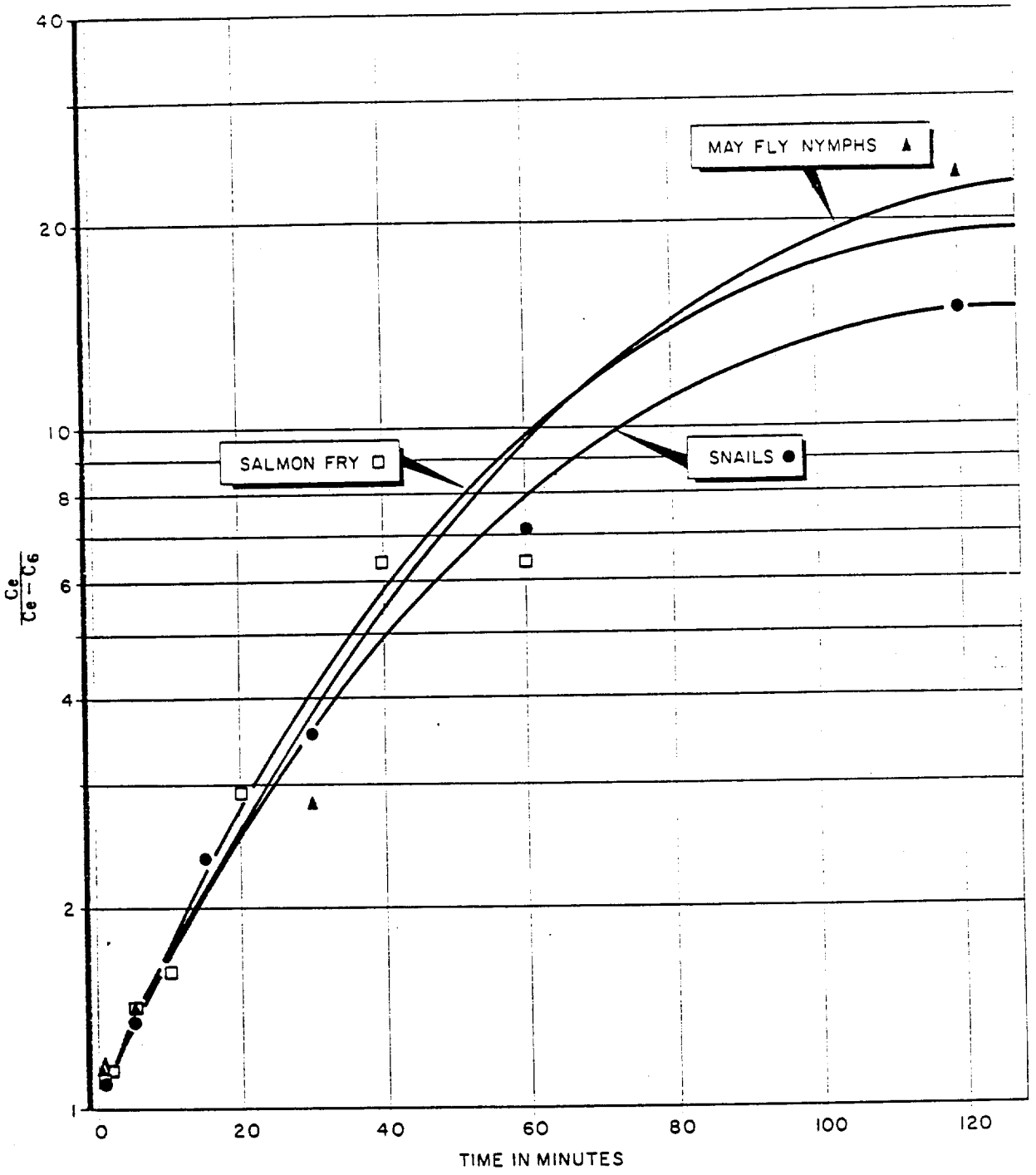

FIGURE I

RATE OF INCORPORATION OF TRITIUM OXIDE INTO BODY WATER

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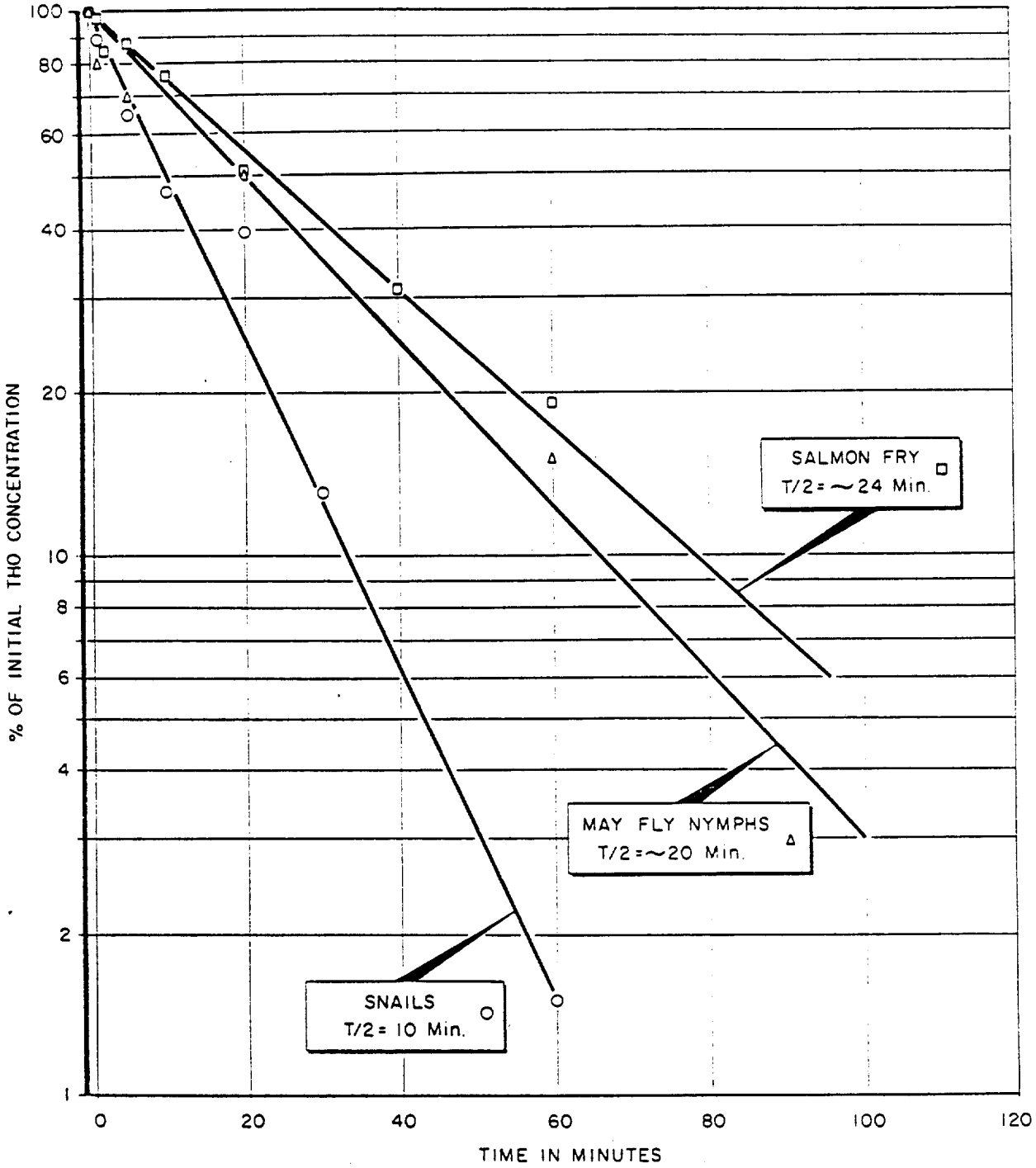

FIGURE 2

RATE OF ELIMINATION TRITIUM OXIDE FROM BODY WATER

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EFFECT OF RADIATION ON THE INCORPORATION OF TRITIUM BY THE RAT

J. E. Ballou and R. C. Thompson

The quantity of tritium oxide administered to rats was shown to have no significant effect on the extent to which such tritium is incorporated into tissue compounds.

In order to study the retention of tissue-bound tritium in rats over long time periods, it is necessary to administer relatively large quantities of tritium oxide which result in appreciable irradiation of the animal. Although no gross evidence of radiation effects was observed with dosage rates as high as 30 rads/day and total doses as high as 550 rads (1), it was considered essential to determine whether such amounts of radiation might affect the rate of tritium incorporation into tissue compounds. In preliminary experiments with small numbers of animals, no such effect was observed in animals sacrificed shortly after injection of varied amounts of tritium oxide. There was, however, a possible indication of such an effect in animals sacrificed at long intervals following multiple injections. The present experiment was designed to follow up this lead.

METHODS

Three groups of six rats each (Sprague-Dawley, females, average wt. 270 g) were given weekly intraperitoneal injections of tritium oxide for a total of three injections each. Quantities of tritium oxide administered in each injection were 25 mc, 5 mc, and 1 mc for the three groups. The radiation dose rate for the highest level group was approximately 30 rads/day following each injection and the total radiation dose received

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by this group was approximately 400 rads. Dose rates and total doses for the other two groups were lower by factors of 5 and 25. All animals were sacrificed 60 days after the final injection and representative samples analyzed for organically bound tritium (2).

RESULTS AND DISCUSSION

A statistical summary of the results from this experiment is shown in Table 1. The considerable variation between animals in a given group precludes the recognition of any small effect of radiation on tritium incorporation and retention. Certainly, within the limits of biological variation, there is no indication of such an effect. The rather large variation noted within groups is not surprising in such a long-term experiment. Small differences between animals in the biological parameters which determine the retention of bound tritium would account for large differences in retention after 60 days.

ACKNOWLEDGMENTS

We wish to acknowledge the technical assistance of Margaret B. Lawson and Alma L. Crosby.

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TABLE 1

Organically Bound Tritium Concentrations
(μc/ml Combustion Water ± Std. Dev.)

Group No.	1	2	3
mc/Injection	1	5	25
Factor by Which Measured Concentrations are Multiplied	25	5	1
Carcass	1.18 ± 0.12	1.01 ± 0.12	0.96 ± 0.14
Liver	0.26 ± 0.06	0.15 ± 0.03	0.19 ± 0.05
Sm. Intestine	0.50 ± 0.11	0.38 ± 0.07	0.50 ± 0.13
Muscle	1.52 ± 0.60	1.36 ± 0.18	1.26 ± 0.23
Pelt	1.15 ± 0.36	1.15 ± 0.24	1.16 ± 0.15
Fat	0.84 ± 0.17	0.92 ± 0.39	0.89 ± 0.25

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TOXICITY OF I^{131} IN SHEEP
XIV. LOW-LEVEL CHRONIC EFFECTS

L. K. Bustad, L. A. George, P. L. Hackett,
S. Marks,* and H. A. Kornberg

This paper reports the progress on a chronic experiment designed to determine the minimum quantity of I^{131} that may be toxic when administered daily to sheep. Original experimental ewes that received 5 and 0.15 μc of I^{131} since 1950 were sacrificed. Definite damage was restricted to histopathological lesions of the thyroid in the 5 μc/day group and a slight depression in PBI and leukocyte count in a few individuals of the original 5 μc/day group.

Papers I to IV of this series (1) presented results obtained during the first 20 months of investigation, papers V to IX (2) summarized the 1952 results and papers, and X to XIII (3), the 1953 results. This paper summarizes the general observations made during 1954 on all remaining animals in the original experimental group, their offspring and the new groups fed 0.5 and 1.5 μc/day.

METHODS

The number of animals and groups under investigation in January 1954 was as follows:

TABLE 1

Number of Animals in Groups at 45 Months

	μc I^{131} Fed/Day				
	5	1.5	0.5	0.15	Control
No. of Original Ewes	6			4	7
" 1950 Offspring	6			6	6
" 1951 and 1952 Offspring	6	6	6	8	6
" 1953 Offspring	3				9

* Consultant in Pathology to Radiological Sciences Department.

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All the ewes remaining in the original control, 0.15 μc/day, and 5 μc/day groups were sacrificed as they reached six years of age in order to determine fetal thyroid weights and I^{131} uptake at various stages of gestation (4).

The following animals and groups were under study in December 1954:

TABLE 2

Number of Animals in Groups at 52 Months

	μc I^{131} Fed/Day				
	5	1.5	0.5	0.15	Control
No. of 1950 Offspring	5			6	6
" 1951 and 1952 Offspring	4	6	5	8	6
" 1954 Offspring	1	5	3		

Weekly external monitoring of all sheep thyroids continued. Periodic blood samples were collected from all ewes. Hematological procedures included leukocyte count, differential count, hemoglobin, and packed cell-volume determination. Blood-chemistry procedures included creatinine, protein-bound iodine, inorganic phosphorus, and urea-nitrogen determinations. In addition, specimens of blood, urine, feces and milk were submitted for assay of I^{131} concentration. Routine sampling of tissues for radioactivity and histologic study was performed at necropsy on all exposed animals and on representative numbers of lambs from all groups at birth and at weaning.

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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 1950 offspring and in Figure 2 for the 1951 and 1952 offspring. The lower Q/q of the 5 μc/day groups, when compared with the 0.15 μc/day groups, is most marked in the first year offspring. A plot of the annual moving averages of the Q/q of a representative ewe from the original 5 μc group showing damage compared with that of a ewe exhibiting questionable damage is shown in Figure 3. There is no change in the thyroid function, as noted by external monitoring, of ewes fed 0.15, 0.5 or 1.5 μc/day.

Changes in Blood Constituents

The mean values of blood constitutents in the 1950 offspring of the 5 μc/day group during a six-month test period are presented in Table 3.

TABLE 3

Blood Constituents (Mean Values) in Four-Year-Old Ewes

Constituent	1950 Offspring		
	5 μc/day Group	Control Group	Avg Difference Between Groups
PBI (μc/100 ml)	2.3	4.2	1.9 ± 0.2 *
Inorganic Phosphorus (mg/100 ml)	4.3	4.3	None
Creatinine (mg/100 ml)	0.93	0.87	0.051 ± 0.046
Urea Nitrogen (mg/100 ml)	22	20	None
Packed-Cell Volume (%)	35	33	1.21 ± 1.20
Hemoglobin (g/100 ml)	10.8	10.6	None
Leukocytes (cells/cmm)	7,700	9,200	1500 ± 500 *
Lymphocytes (cells/cmm)	4,300	5,400	1100 ± 400 *
Neutrophils (cells/cmm)	3,300	3,700	None

* 99% Confidence Limits

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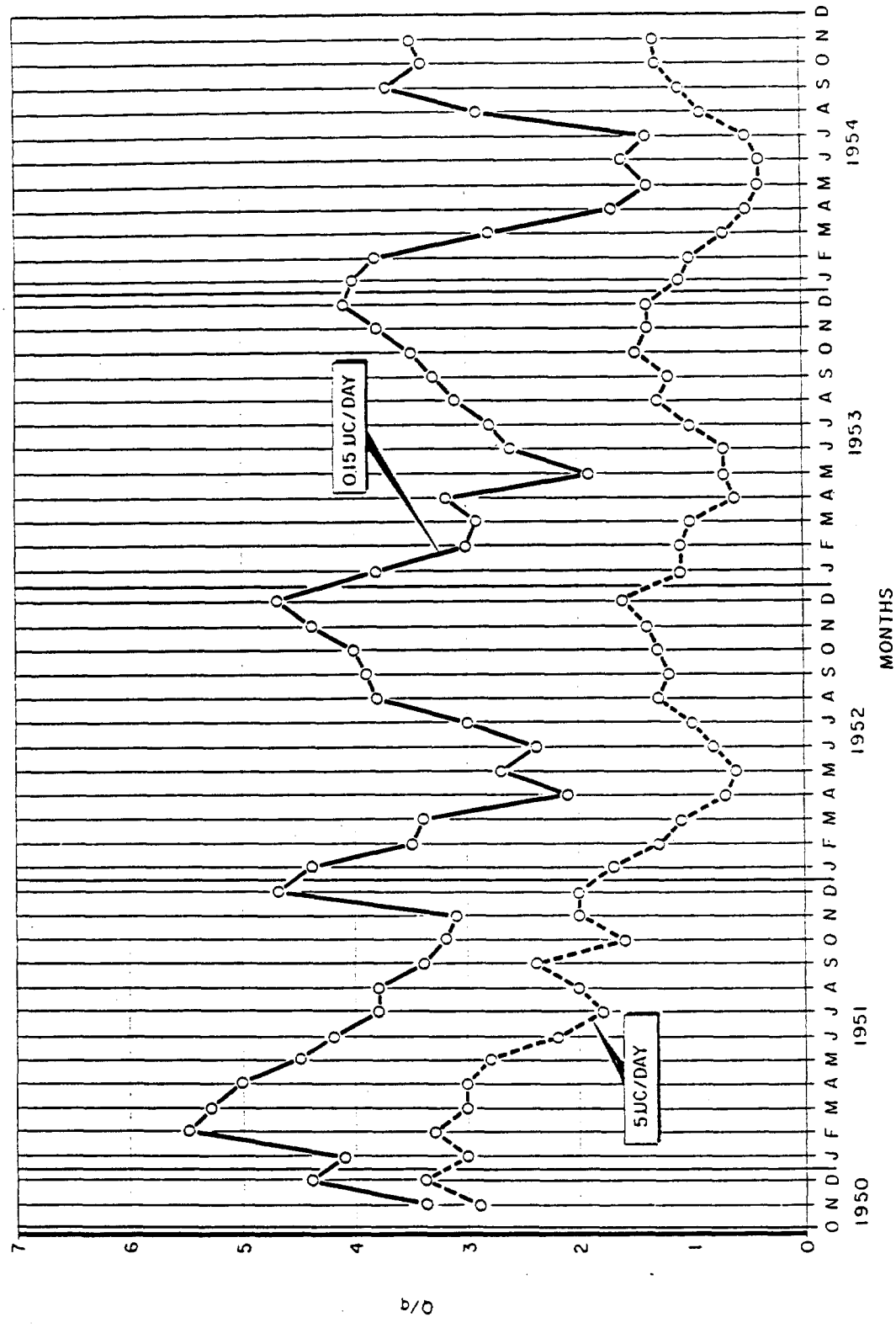

FIGURE I

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

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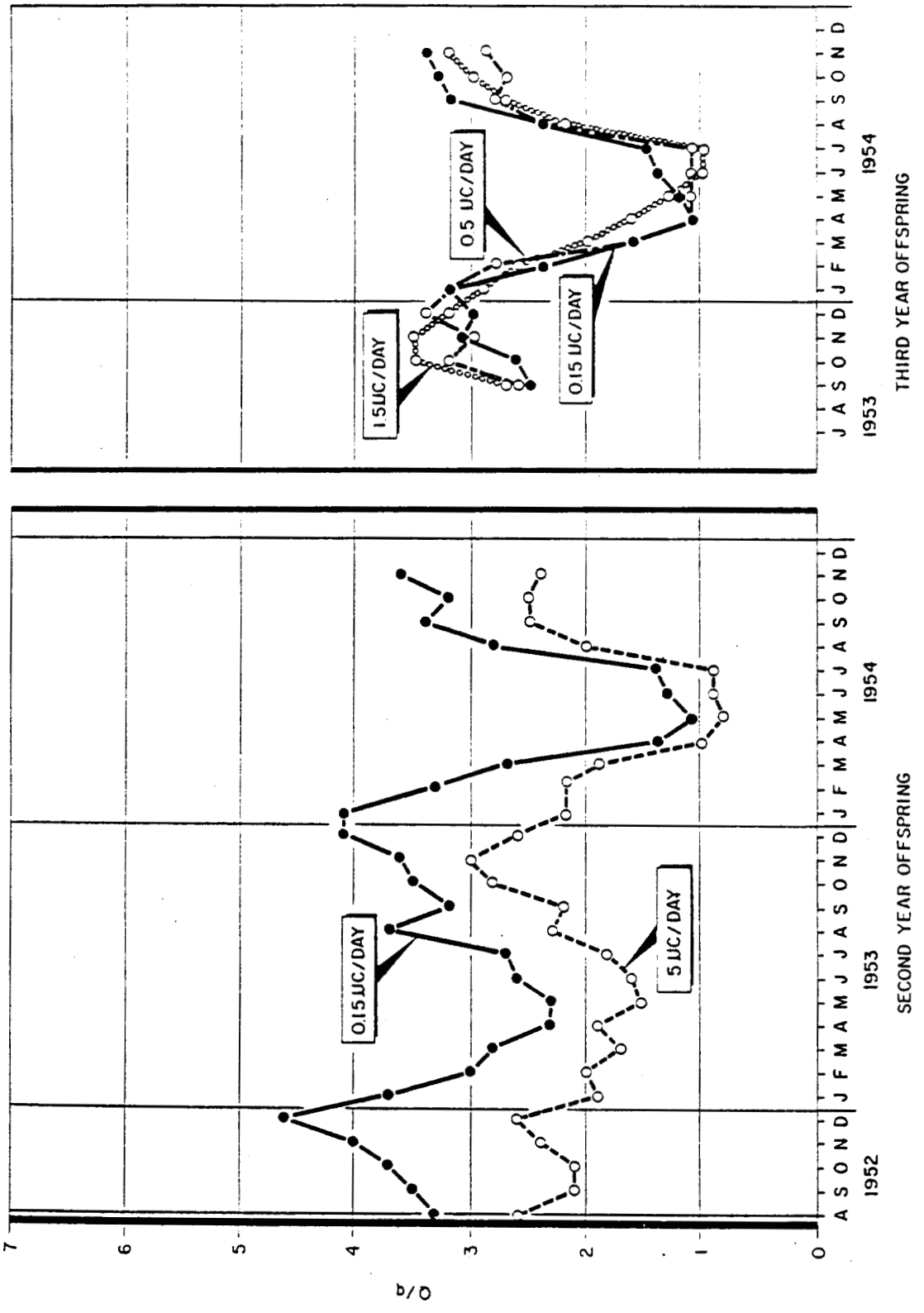

FIGURE 2

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

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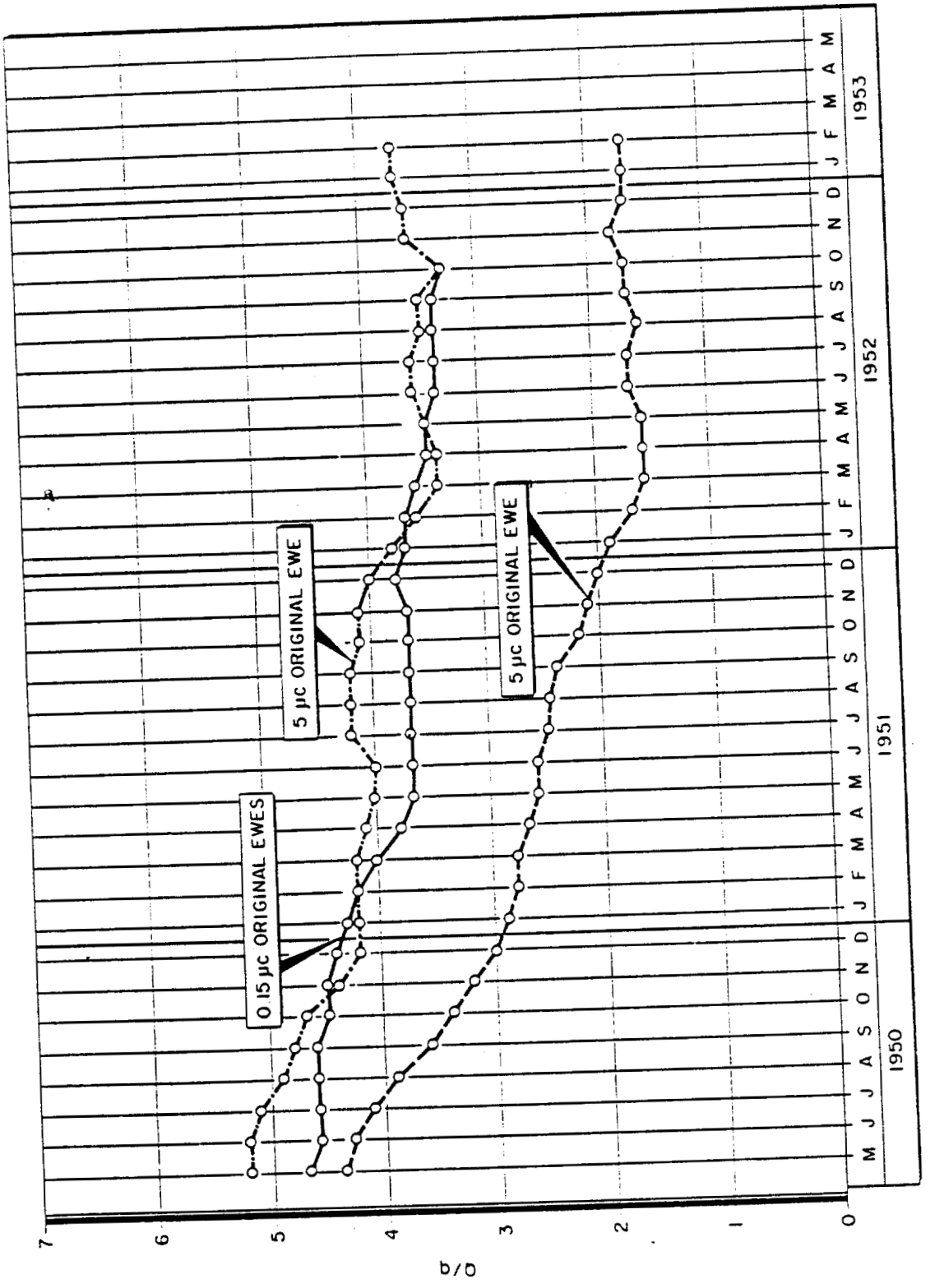

FIGURE 3

MOVING AVERAGES OF RATIO OF I^{131} IN THYROID/I^{131} FED DAILY

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The depression in PBI, and leukocyte and lymphocyte counts noted in Table 3 are considered biologically significant. The mean value for protein-bound iodine, although depressed in the experimental group, is considered in the lower range of normal and not significantly indicative of a hypothyroid condition. Only a few of the members of the original 5 μc/day group showed a depression in PBI and leukocyte count.

Necropsy and Histological Findings

Significant gross-pathological findings were observed only in the 5 μc/day group of sheep and were restricted to the thyroid gland. The involved thyroid glands showed an increase in connective tissue and were reduced in size. The mean thyroid weight of the 5 μc/day ewes was 5.3 ± 2.7 g compared with control thyroid weights of 16 ± 4.1 g. The large size of the undamaged glands is attributed to a marginally goitrogenic diet (1), the I^{131} administered being carrier-free. The estimated dose to the thyroid gland in the 5 μc group was 19,000-35,000 rads over the 30-46 months of I^{131} feeding.

Six thyroid glands from the remaining original 5 μc adult animals were examined histologically during 1954. Five glands showed definite damage, and the findings were considered questionable in the sixth. Fibrosis between the follicles was the consistent manifestation of damage in this group. The effects were usually of minimal degree although one thyroid showed moderate fibrosis. The follicles displayed a general reduction in size. Minimal changes were also observed in occasional arteries, the effects consisting of intimal fibrosis, distortion of the endothelial cells and fraying of the internal elastic lamina. The arterial damage was observed most frequently in vessels lying immediately external to the capsule of the gland.

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I^{131} in Tissues and Excretions

The concentrations of I^{131} in various parts of the 5 μc ewes at necropsy are recorded in Table 4.

TABLE 4

Mean Concentration of I^{131} in 5 μc Ewes at Necropsy
(In Units of 10^{-4} μc/g)

Thyroid	15,000
Abomasal content	14
Mandibular salivary gland, urine, feces, milk	4-8
Parotid salivary gland	4
Cecum content	4
Liver	3
Adrenal	3
Pituitary	3
Kidney	3
Abomasal wall	3
Bile	3
Bladder wall	2
Ovary	2
Lung	2
Heart	2
Blood	2
Spleen	<2
Brain	<2
Wool	<2

The study is continuing to determine within a factor of three the quantity of I^{131} that may be ingested daily without causing damage to sheep during their normal life span. The animals now receiving 0.5 and 1.5 μc/day are expected to furnish these data.

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ACKNOWLEDGMENTS

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

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TOXICITY OF I^{131} IN SHEEP

XV. I^{131} Uptake by Fetal Thyroid During Various Stages of Gestation

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

The I^{131} concentration of the fetal thyroid gland was found to increase exponentially during the second and third trimesters of pregnancy. The total I^{131} in the fetal thyroid approximated the daily dose of I^{131} administered to the dam in advanced pregnancy. At the same period the I^{131} concentration in the fetus exceeded that of the dam.

A previous paper in this series (1) reported that uptake of I^{131} by the fetal thyroid of the sheep begins at about 50 days of gestation. This experiment was designed to determine the I^{131} concentration in fetal thyroids during the balance of the gestation period of about 95 days.

METHODS

Three groups of adult ewes, six ewes per group, maintained on a marginal goitrogenic diet were used in the experiment. One group (control) received no I^{131}, while the other groups received, respectively, 0.15 and 5 μc of I^{131} per day for more than 42 months. Cesarian sections were performed on one animal of each group at 55, 70, 85, 100, 115, and 130 days of gestation. Autopsies were performed on recovered fetuses and a complete radioanalysis of tissues was made. Weights and sizes of the fetuses and the principal body organs were recorded.

RESULTS AND DISCUSSION

Thirty fetuses were recovered from the eighteen ewes. The growth rate of the fetuses is recorded in Figure 1. Values obtained were slightly higher than those reported by Barcroft (2) and may be attributed to the

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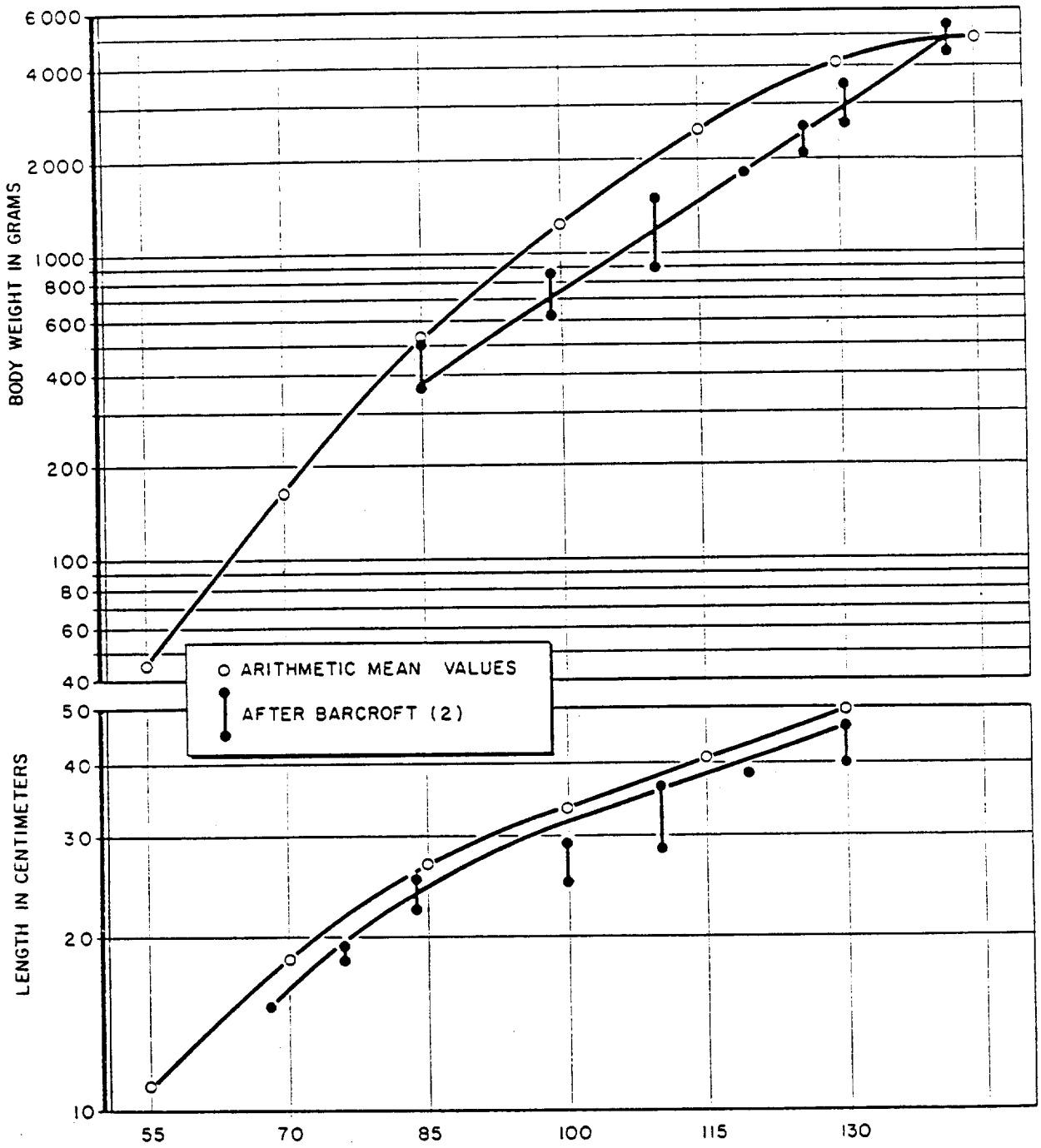

FIGURE 1

FETAL GROWTH

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high nutritional plane maintained in the experimental animals. Thyroid growth rate is compared with that of certain other organs in Figure 2.

Radiochemical analysis of the fetal thyroid gland demonstrated an exponential rise in I^{131} thyroid content with fetal age (Figure 3). The high I^{131} concentration in the 85-day fetal thyroid of the 5 μc/day group (Figure 3) may be partially explained by the relatively low concentration found in the injured thyroid of the dam. Pathological examination of the adult thyroids indicated a varying degree of radiation damage (3).

The fetal thyroid I^{131} concentration in relation to the daily maternal dose appears in Table 1.

TABLE 1

Concentration of Radioiodine by the Fetal Thyroid
(Expressed as Percentage of Dam's Daily Dose)

	Days in Gestation					
Group	55	70	85	100	115	130
5 μc/day	0.1*	1.1	12*	31*	58	110*
0.15 μc/day	0.1*	1*	4*	32*	79*	94*

* Average of twins.

Comparative concentrations of I^{131}/g of fetal and maternal thyroid glands are shown in Table 2.

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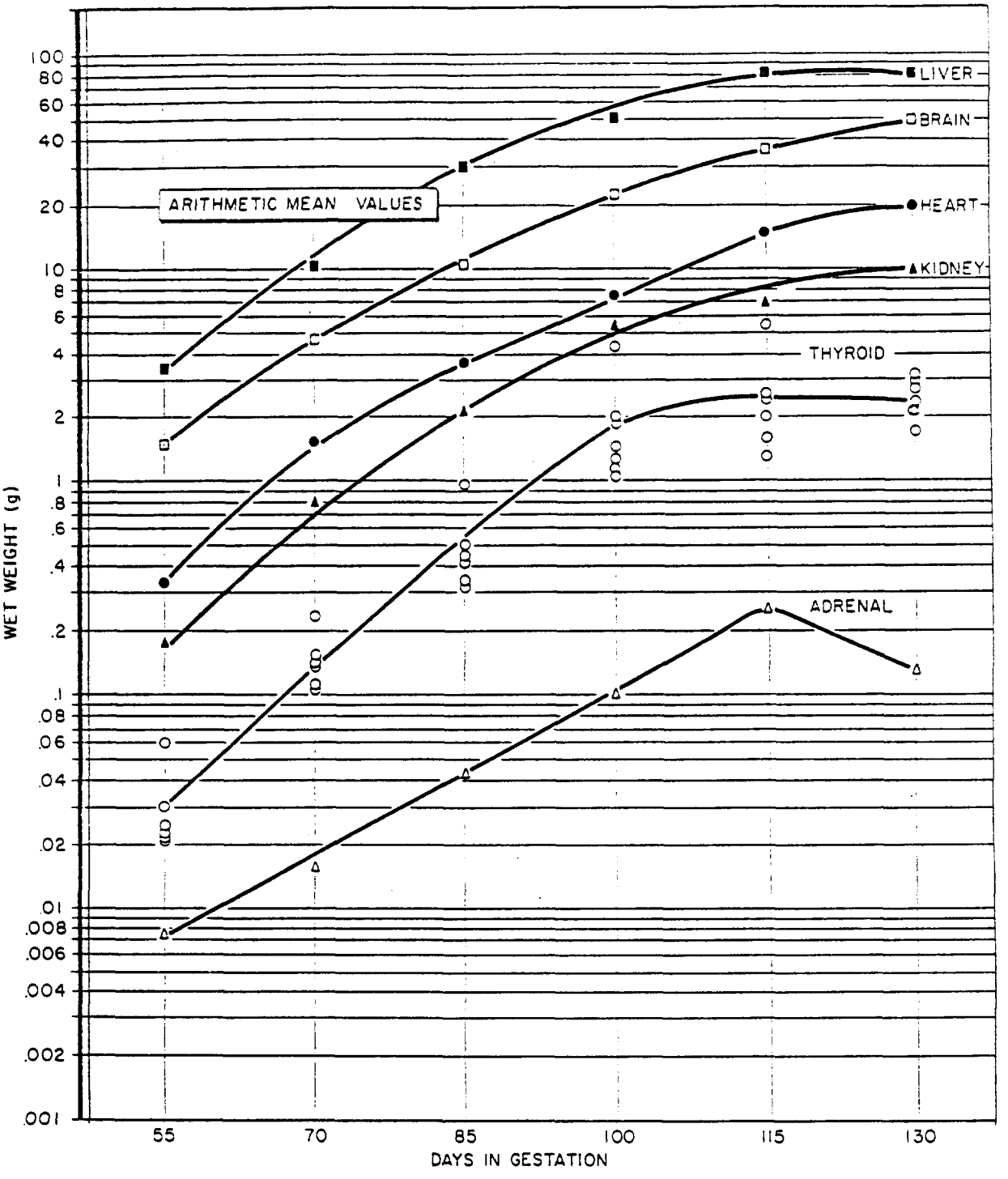

FIGURE 2

FETAL ORGAN WEIGHTS

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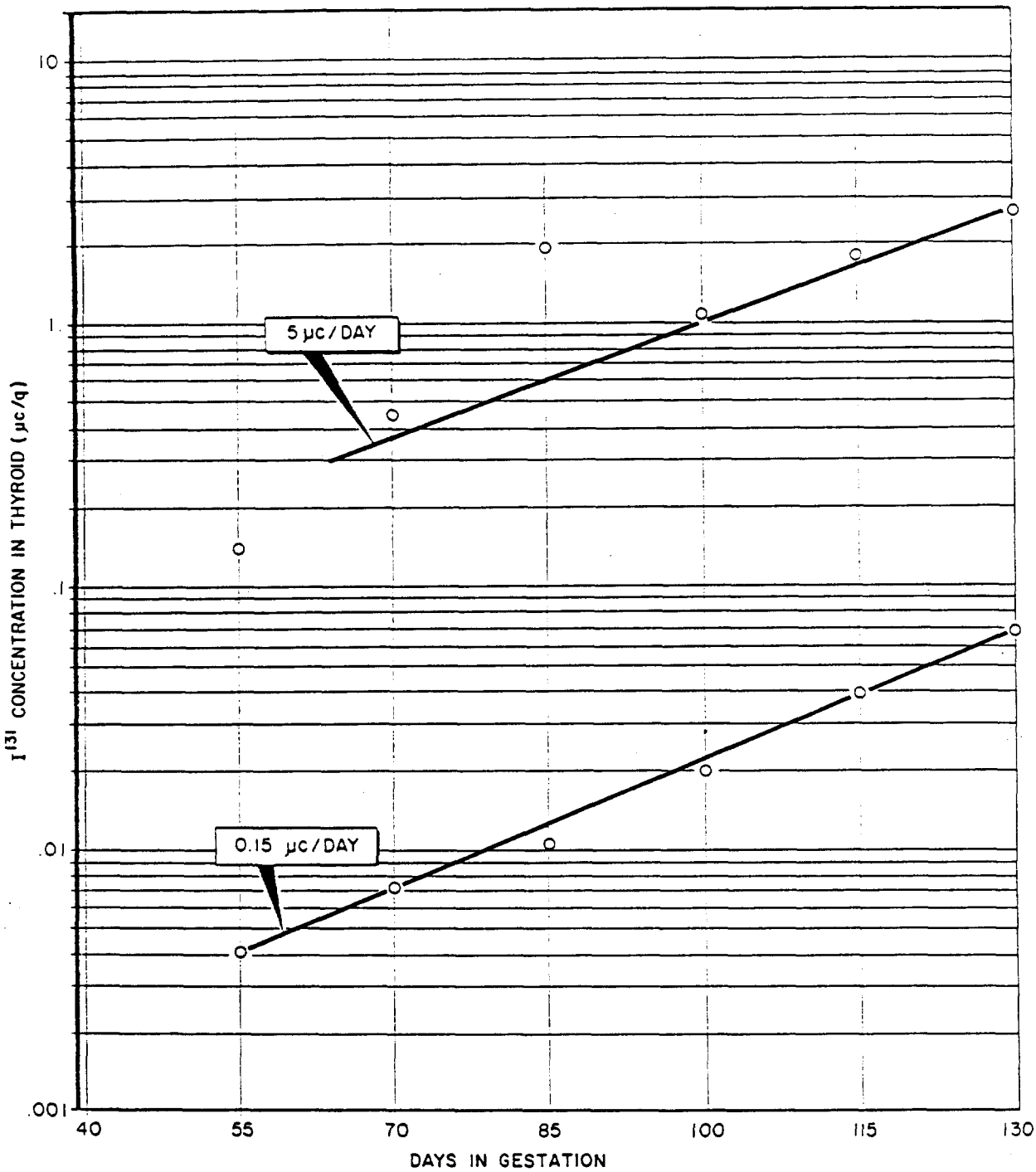

FIGURE 3
CONCENTRATION OF RADIOIODINE IN FETAL THYROIDS

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TABLE 2

Concentration of I^{131} in Maternal and Fetal Thyroids (μc/g)

Gestation Age at Sacrifice	0.15 μc/day		5 μc/day	
	Dam	Offspring	Dam	Offspring
55	0.023	0.004 .005	0.8	0.14
70	.044	.008 .007	3.0	.5
85	.034	.006 .013	.8	1.9 2.0
100	.028	.013 .026	1.1	.8 1.5
115	.028	.050 .032	1.0	1.8
130	.028	.08 .05	2.6	2.6 2.8

The concentration of I^{131} in the fetal thyroid is one to two times that of the dam's thyroid by 115 days gestation. The partition of I^{131} between the thyroids of the dam and the fetus indicated that the fetal thyroid is the critical organ in assessing I^{131} toxicity if a contamination event occurs in advanced pregnancy.

ACKNOWLEDGMENTS

The assistance of M. E. Kerr and personnel of the Animal Farm Staff and the Biology Control Unit is gratefully acknowledged.

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TOXICITY OF I^{131} IN SHEEP
XVI. BIOLOGICAL COEFFICIENTS ASSOCIATED WITH I^{131} METABOLISM BY THYROID

H. A. Kornberg, G. E. Pilcher*, H. T. Norton*, L. A. George, Jr. and L. K. Bustad

Two experiments are described which were performed in order to test the theoretical values that are applied in calculation of the hazard to animals following a single I^{131} contamination event. Mathematical and statistical analyses were conducted on measurements of I^{131} concentrations in the thyroid gland following both a single tracer study and chronic feeding. The functional relationship shows good agreement with experimental findings.

Mathematical and statistical analyses were performed on measurements of I^{131} concentrations in the thyroid gland of sheep resulting from single and chronic feeding experiments. These measurements were studied in order 1) to determine the biological coefficients associated with I^{131} ingestion, 2) to compare the values of these coefficients in the two types of feeding, and 3) to compare their application in determining hazards to grazing animals following a single contamination event.

METHODS

In the single dose experiment, 15 μc of I^{131} was orally administered to each of three rams which had been maintained on a marginally goitrogenic diet. The amounts of I^{131} in the thyroid glands were determined during the subsequent 21-day period by external monitoring with a specially built sheep-neck monitor containing three Geiger-Mueller tubes (1). In the chronic feeding experiment, hay which constituted the entire ration of the same three rams was contaminated to a level of 8.4×10^{-4} μc/g on the first day of feeding. The contaminated hay was then fed to the animals in known amounts and their thyroids were externally monitored daily for 41 days.

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* Members of Biophysics Section.

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RESULTS AND DISCUSSION

Single I^{131} Administration

Following absorption by the thyroid, the quantity of radioiodine in the gland at any time is dependent only on the physical and biological half-life of the administered iodine. Maximum absorption by the thyroid was found to occur two to three days after the I^{131} was orally administered. The mathematical expression defining the quantity, Q (in μc), in the organ at t days following a tracer contamination is

$$Q = aq_o e^{-(\beta + \lambda)t},$$

where a is the decay-corrected fraction of the dose which reaches the gland, q_o is the quantity of I^{131} (in μc) administered, β is the biological decay constant, and λ is the physical decay constant.

Chronic I^{131} Administration

When the radioiodine is administered continuously from a decaying source, the net rate with which the thyroid takes up the substance may be expressed by

$$\frac{dQ}{dt} = aq_o e^{-\lambda t} - (\beta + \lambda) Q,$$

where the symbols denote the same quantities as indicated above. From this,

$$Q = \frac{aq_o e^{-\lambda t}}{\beta} \left[1 - e^{-\beta t} \right].$$

If a new quantity P is defined as $Qe^{\lambda t}$,

$$P = \frac{aq_o}{\beta} \left[1 - e^{-\beta t} \right].$$

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Differentiation of this equation with respect to t gives

$$\frac{dP}{dt} = aq_o e^{-\beta t}.$$

Taking the natural logarithm of this equation yields $\ln \frac{dP}{dt} = \ln aq_o^{-\beta t}$. From this equation the biological parameters can be determined from the experimental data by the method of least squares regression. The value of q_o was determined independently through the measurement of the initial concentration of I^{131} in the contaminated hay and the daily quantity of hay ingested. When values for coefficients so obtained were used in the expression for Q above to plot a curve of Q versus t, only a poor fit with the experimental points was obtained.

However, when the experimental data were extrapolated to zero thyroid activity, a value of $t = 1.8 \pm 0.2$ days was found. Using this factor, a revised expression for Q was obtained:

$$Q = \frac{aq_o e^{-\lambda t}}{\beta} \left[1 - e^{-\beta(t - 1.8)}\right].$$

The equation is plotted in Figure 1 and appears to meet the requirements of the experimental data.

Biological Coefficients Measured

In Table 1 are listed the coefficients obtained from the single and chronic feeding experiments. Values for the biological half-lives of the I^{131} in the chronic experiment were assumed to be the same as those obtained in the acute experiment.

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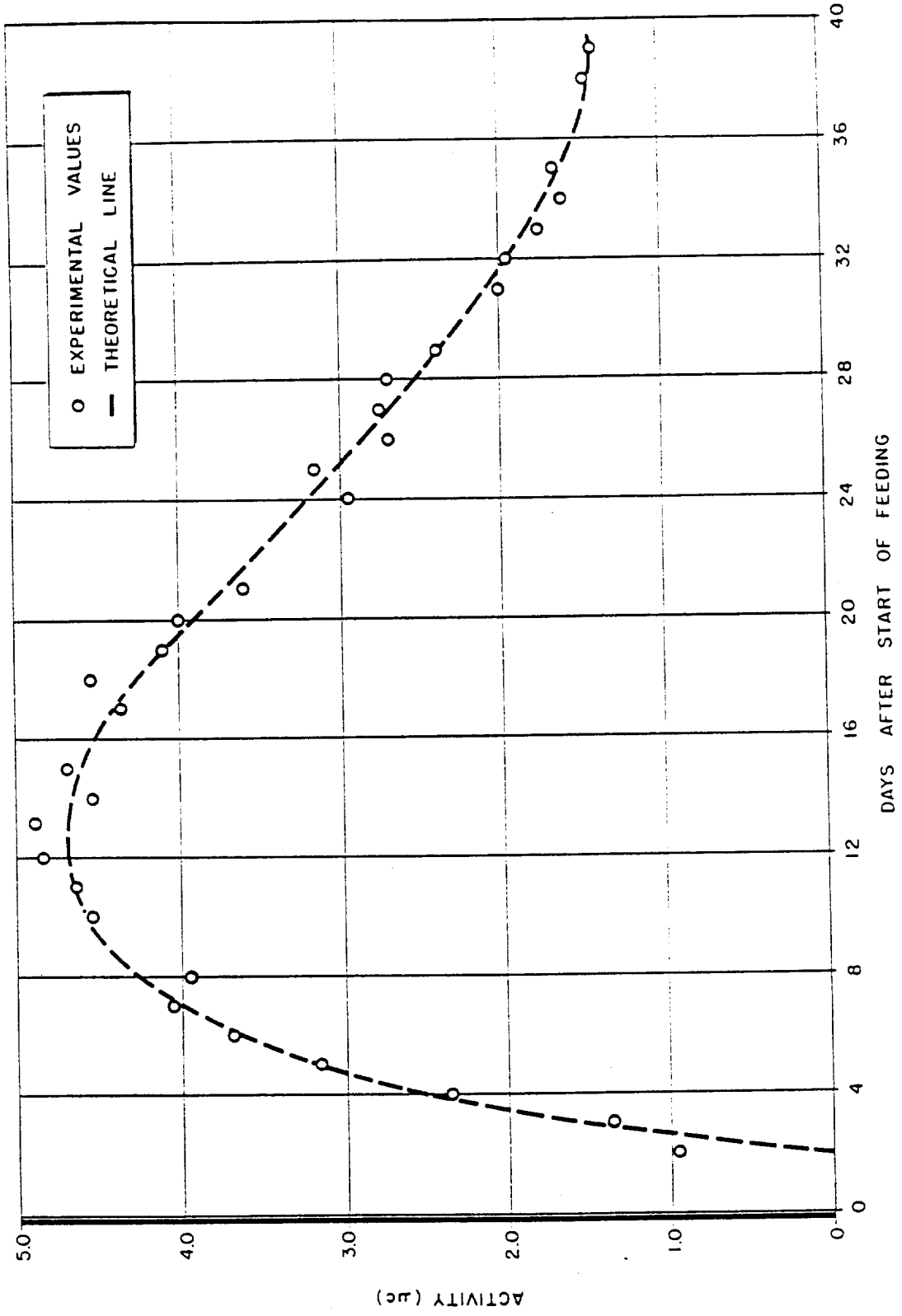

FIGURE I

I^{131} ACTIVITY IN THYROID GLAND OF SHEEP (CHRONIC FEEDING)

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TABLE 1

Half-Life and Absorption Coefficients

Sheep No.	Single Administration		Chronic Administration
	T 1/2 (days)	a	a
1	34	0.52	0.56
2	28	0.58	0.54
3	37	0.51	0.51

ACKNOWLEDGMENTS

The work of C. M. Barnes, M. E. Kerr and A. F. Wood in the care, feeding and handling of animals, and of Zane Carey and associates in Biophysics Section for radiochemical analysis is gratefully acknowledged.

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TOXICITY OF I^{131} IN SHEEP

XVII. Acute Toxicity of I^{131} in Sheep

L. K. Bustad, R. J. Morrow*, P. L. Hackett, S. Marks**, and L. A. George, Jr.

The effects of single doses of 5 and 15 mc of I^{131} are described. Depression in lymphocyte count, serum, and protein-bound iodine values and reduced effective I^{131} half-life in the thyroid were observed in the 15 mc level only. Pathologic damage was moderately severe after 5 mc of I^{131} and extensive necrosis occurred after 15 mc.

In order to compare the effects of acute and chronic administration of I^{131} an experiment was performed in which single doses of 5 or 15 mc of I^{131} were administered to each of eight animals. The doses chosen were intended to approximate the threshold range for the development of damage in order to permit a comparison with data obtained in the daily administration of a chronic threshold toxic level.

METHODS

In addition to the animals given a 5 or 15 mc dose of I^{131}, four ewes received single doses of 5 μc in order to obtain a thyroid uptake curve typical of a tracer dose. Table 1 gives the number of animals, the amounts administered, and the time of sacrifice.

TABLE 1

Experimental Design

Dose	15 mc	5 mc	5 μc
Number of Animals	4	4	4
Time of Sacrifice after I^{131} Administration (days)	7, 15, 30, and 60	7, 15, 30 and 60	

* Separations Section, Manufacturing Department.
** Consultant in Pathology to Radiological Sciences Department.

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External thyroid monitoring was performed using an ion chamber except in the 5 μc group where a special collar containing three Geiger-Mueller tubes was utilized (1). The monitoring frequency for the low-level group was once each day, while for the other two groups the animals were monitored twice daily for three days, daily for ten days, and then every two days for sixteen days.

Blood samples for hematological, chemical, and I^{131} determinations were taken daily for the first four days, twice during the following week, and then weekly.

Representative animals in each group were placed in metabolism cages to collect total excretions of urine and feces which were submitted for radiochemical analysis.

At necropsy tissues were taken for routine radiochemical analysis, and thyroid tissue was submitted for histopathological study.

RESULTS AND DISCUSSION

I^{131} Absorption and Turn-over

The percentages of I^{131} that appeared in the thyroids of one animal from each of the three groups are shown in Figure 1.

The effective half-life of I^{131} in the thyroid and the maximum absorption coefficient (highest concentration of I^{131} in the thyroid/dose administered) are listed in Table 2.

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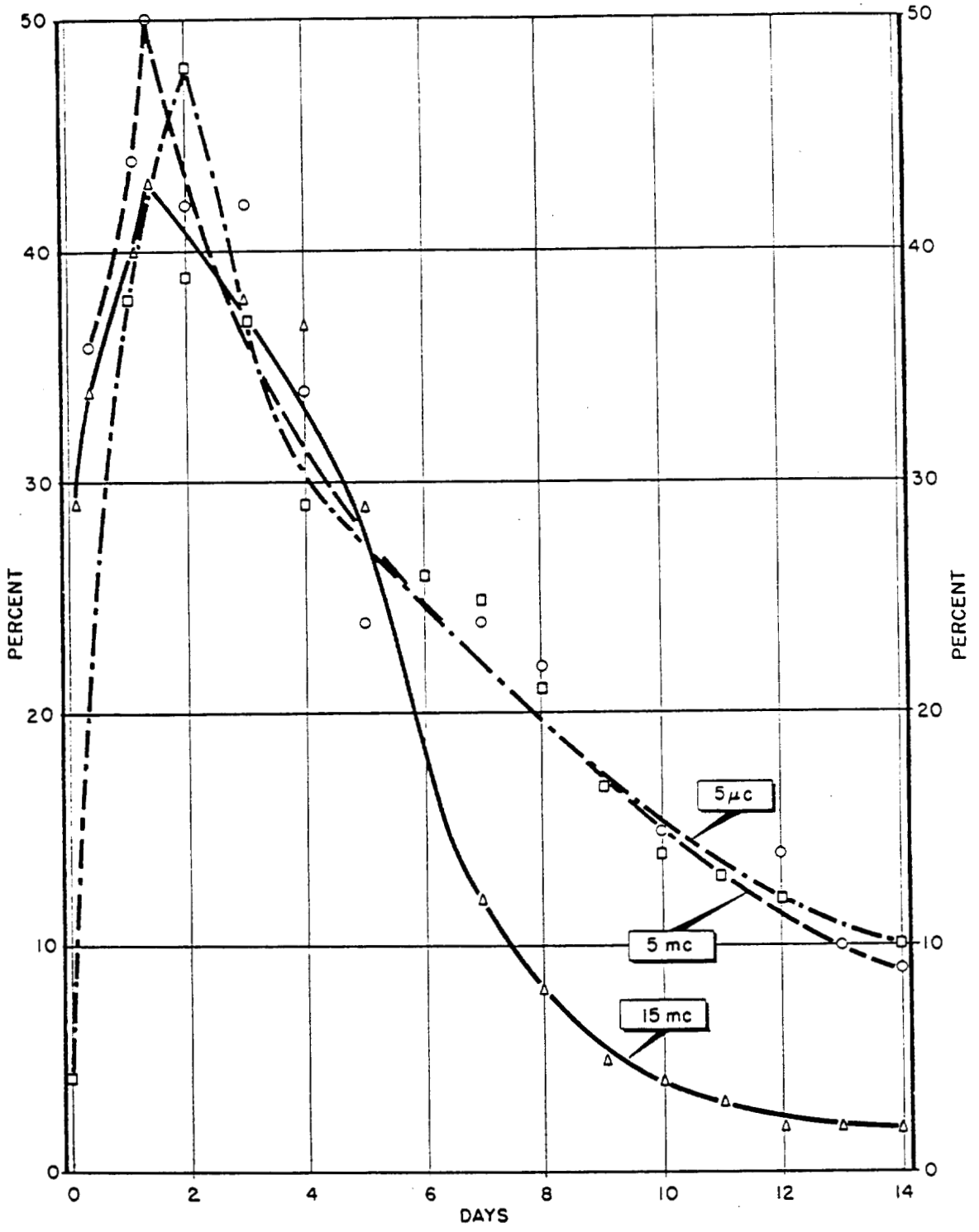

FIGURE I
PERCENT OF ADMINISTERED DOSE IN THYROID IN REPRESENTATIVE ANIMALS

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TABLE 2

I^{131} Half-Life and Absorption in Thyroid

	15 mc group	5 mc group	5 μc group
Effective T 1/2 (in days)	1.7-6	5-6	5-7
Absorption Coefficient (max)	0.41-0.43	0.38-0.50	0.37-0.48

The estimated dose to the thyroid in each animal appears in Table 3.

TABLE 3

Estimated Radiation Exposure of Thyroids

mc Fed	Days Exposed	Total Dose in Rads
5	7	14,000
5	15	16,000
5	30	30,000
5	60	36,000
15	7	33,000
15	15	60,000
15	30	75,000
15	60	60,000

The apparent inconsistencies in the dose to the thyroid gland are attributed principally to differences in absorption coefficients, size of thyroid glands, and the effective half-life of thyroidal I^{131}.

Changes in Blood Constituents

Determinations included protein-bound iodine, cholesterol, inorganic phosphorous, creatinine, urea nitrogen, total protein, packed cell volume, hemoglobin, erythrocyte count, leukocytes, and differential count. The only change was evidenced by the 15 mc group and was restricted to a change in the serum protein-bound iodine (PBI), as illustrated in Figure 2,

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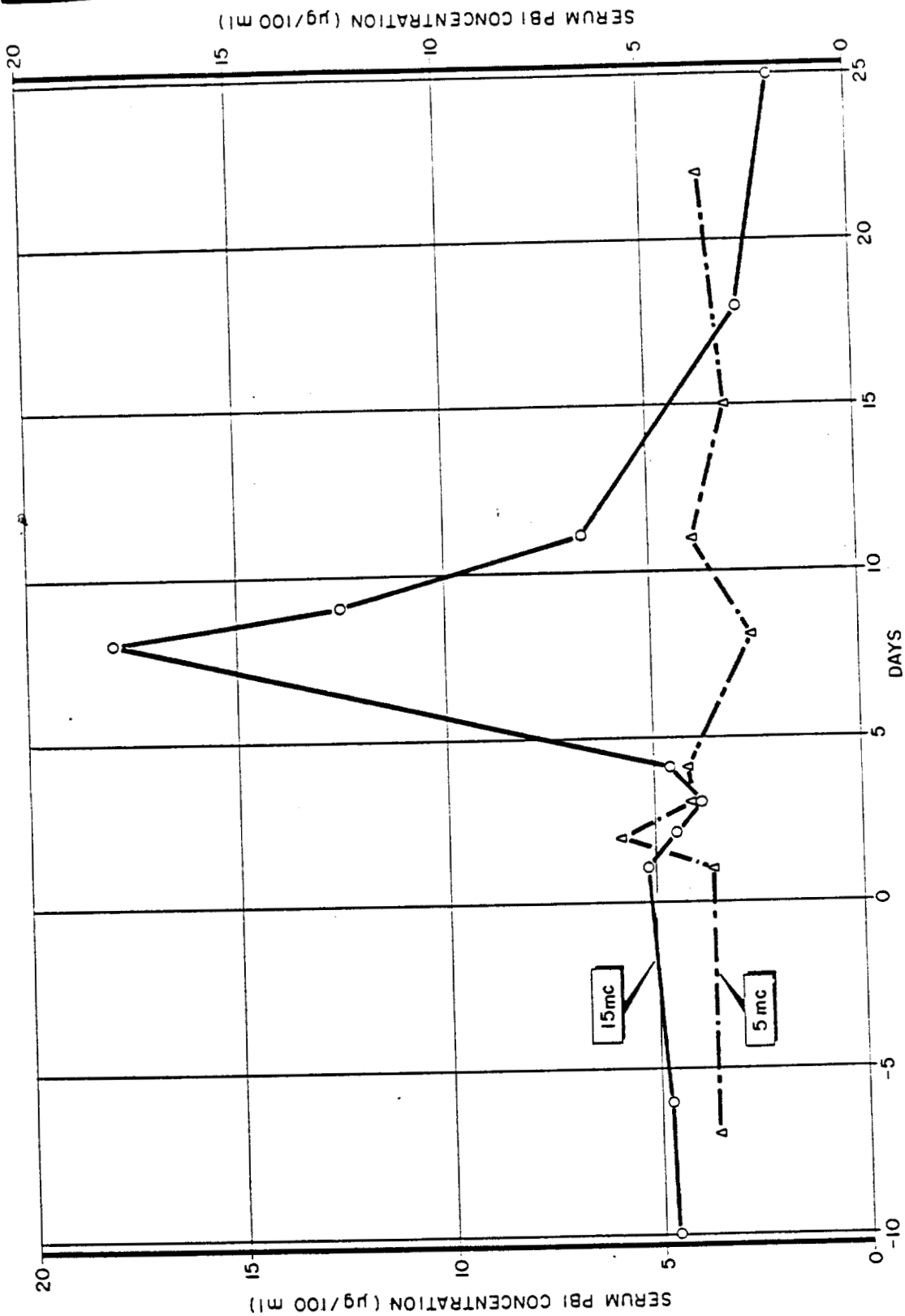

FIGURE 2

SERUM PBI CONCENTRATION IN ANIMALS FED 5 & 15 mc I^{131}

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and a depression in the lymphocyte count (Figure 3). The animal whose PBI rose abruptly on the seventh day after the I^{131} administration showed a slight PBI depression prior to sacrifice (Figure 2). A sudden rise of PBI following high acute doses of I^{131} was described by Riggs (2) in man and by Feller et al. (3) in rats. Tong et al.(4) in making similar observations in rats in 1952 attributed the sudden elevation of PBI to thyroglobulin or some similar protein being released from a severely damaged thyroid. The sudden elevation noted in our experiment was striking in only one of four animals fed 15 mc of I^{131}.

Thyroid Histopathology

Severe damage to the thyroid was present in both series of animals, the effects being more pronounced in the 15 mc group.

The first animal in the 5 mc group, which was sacrificed at the end of 7 days, showed no evidence of pathologic effects in the thyroid other than intimal thickening due to fibrosis and endothelial hyperplasia in a few arteries. At 15 days, mild, loose fibrosis and chronic inflammation were observed in the interstitial tissue. The fibrosis and inflammation were more extensive at 30 days, and the lumens of follicles contained desquamated epithelial cells as well as invading stromal cells and neutrophilic leukocytes. Severe damage was present at 60 days with central necrosis and a foreign body giant cell reaction. In addition to the presence of focal dense infiltrations of lymphocytes within the gland, inflammation was encountered immediately external to the gland, one area showing numerous eosinophils.

At seven days, 15 mc of I^{131} (about 33,000 rads) caused no manifest parenchymatous damage. However, small groups of lymphocytes were encountered in the neighborhood of islands of stratified squamous epithelium and may have constituted a response to damge of the stratified squamous epithelium in advance of an effect on the thyroid parenchyma. A moderately

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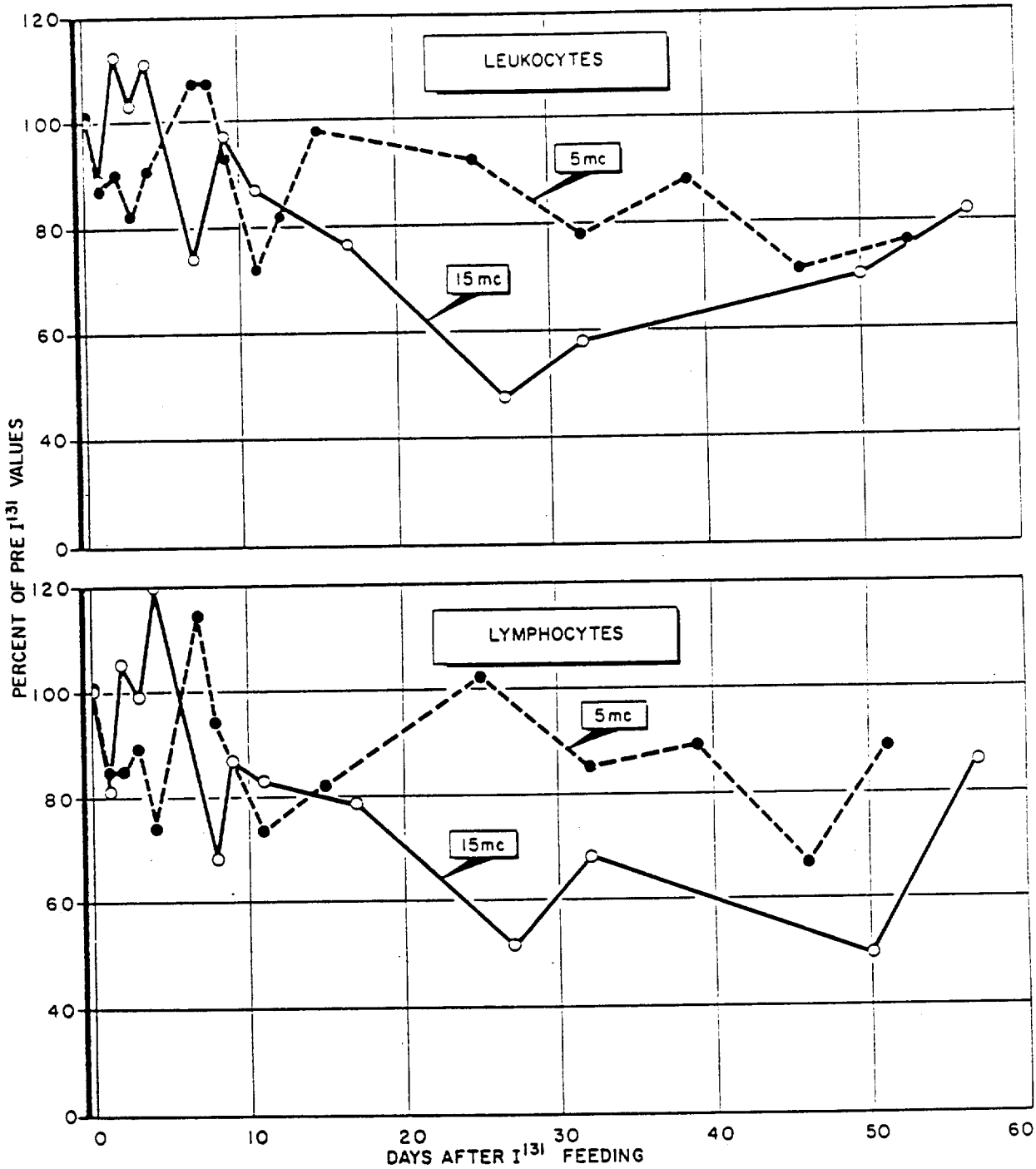

FIGURE 3

LYMPHOCYTE AND LEUKOCYTE COUNTS IN ANIMALS FED 5 AND 15 mc I^{131}

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large artery showed endothelial hyperplasia with neutrophilic leukocytes in the innermost portion of the thickened intima. The greater part of the thyroid gland was necrotic at 15 days. Inflammation in the form of infiltrations of lymphocytes, neutrophils and fewer large mononuclear cells was observed to a marked degree external to the gland and only mildly in the peripheral layer of surviving parenchyma. An interesting incidental feature immediately external to the gland was the presence of an area of thymic tissue which had undergone a substantial depopulation of lymphocytes.

At thirty days, 15 mc (about 75,000 rads) resulted in necrosis similar to that observed at 15 days. In addition, arteries of various sizes showed invasion of both the intima and media by neutrophils, the acute inflammatory reaction causing substantial intimal thickening (Figure 4). Endothelial hyperplasia and fibrosis were responsible for a generalized narrowing of arterioles. The thyroid gland obtained at 60 days disclosed survival of only an intermittent single row of small, damaged follicles in the periphery. The interesting feature at this time was the advent of a reparative process without, however, any evidence of epithelial regeneration. Several foci of intense fibroblastic activity were observed, principally in the periphery of the necrotic zone. Capillaries fanned out from these areas into the necrotic tissue, which had also been invaded by a moderate number of scattered macrophages and focal groups of foreign body giant cells. The appearance was suggestive of an early stage of replacement of the necrotic tissue by fibrous tissue, an event which apparently supervened upon the decay of radioactivity to a level compatible with cellular activity.

Pathologic observations suggest that the thyroid gland would be essentially destroyed by 15 mc while the gland in an animal receiving a single 5 mc dose would still possess much functional tissue.

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Figure 4
Acute inflammation in small artery in thyroid gland sacrificed 30 days after administration of 15 mc of I^{131}. 167 X

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ACKNOWLEDGMENTS

The assistance of M. E. Kerr and Experimental Animal Farm Staff, personnel of the Clinical Laboratory, and N. L. Dockum and E. Jane Coleman is gratefully acknowledged.

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TOXICITY OF I^{131} IN SHEEP
XVIII. HISTOLOGIC PATTERNS IN THE THYROID GLAND AT VARIOUS LEVELS OF I^{131} ADMINISTRATION

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

Comparative patterns of histopathologic damage in the thyroid gland of the sheep are briefly described for feeding levels of 5, 15, 30, 45, 135, 240, 480 and 1800 μc of I^{131} per day. The effects were mild at 5 μc/day and progressed to almost complete epithelial destruction at 240 μc/day.

Studies on animals of the original group administered I^{131} chronically since 1950 have provided sufficient pathologic material to permit a comparison of histologic patterns in the thyroid gland at various feeding levels. This report will briefly discuss the comparison of the histopathology in the various groups.

METHODS

Necropsy and tissue procedures were described in previous papers of this series (1). The numbers of animals included in the analysis of the pathology of the thyroid are presented in Table 1.

TABLE 1

Number of Animals in Various Feeding Levels

μc I^{131} Fed/Day	Control	5	15	30	45	135	240	480	1800
Adults	16	9	3	6	0	0	10	16	3
Offspring	52	18*	9	6	5	7	27	0	4

* Does not include 39 lambs sacrificed at or before 4 months of age.

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RESULTS

A definite variation in the manifestations of thyroid tissue response to I^{131} was observed in relation to the daily dose level and the age at which exposure was initiated.

The typical features of pathologic damage at the lowest toxic level of 5 μc per day were observed in the interstitial tissue and consisted of fibrosis in the adult and edema in the offspring (2). Both effects showed progression from the earliest marginal involvement (about 10,000 rads in 8 months including fetal exposure) to a mild degree of severity (over 30,000 rads in over 43 months). At a later stage in the offspring, slight inflammation and fibrosis supervened (about 19,000 rads in 15 months). Epithelial changes were restricted to the occasional enlargement of nuclei. At feeding levels of 15, 30, 45 and 135 μc various combinations of edema, chronic inflammation and fibrosis occurred (minimum of 10,000 rads in lambs to maximum of over 80,000 rads in adults) with a tendency for the edema to predominate in the offspring and fibrosis to predominate in the adult.

The offspring of ewes receiving a daily feeding of 240 μc per day showed early necrosis and inflammation with fibrosis occurring as a later development (1). The estimated dose to the thyroid gland during fetal life plus the nursing period of 4 months was 70,000 rads. The fibrosis progressed to largely replace the parenchyma and severely constrict the follicles. Changes in cellular size and coarsening of the chromatin pattern in the nuclei were widespread in the severely damaged glands. Similar fibrosis and epithelial atrophy developed in the 240 μc adults after 6 months (about 100,000 rads).

A study of early changes in adult animals receiving 480 μc per day showed that localized foci of infiltration of lymphocytes and neutrophils constituted the first evidence of damage after 19 days (about 18,000 rads)

of feeding (3). Degenerating follicles were present within the areas of inflammation. At 36 days (about 25,000 rads) fibrosis developed and at 43 days (more than 50,000 rads) infarcts were observed. Ultimately, this feeding level of I^{131} caused widespread necrosis with a foreign body giant cell tissue response to the necrosis.

Administration of 1800 μc of I^{131} per day for 420 days (more than 150,000 rads) in the adult caused obliteration of the parenchyma apart from the survival of a few scattered cellular remnants. The epithelial tissue was replaced by a hyalinized fibrous scar. Fetuses exposed to this dose through the mother during the third trimester of pregnancy revealed severe damage, including necrosis, fibrosis and evidence of hemorrhage.

Vascular effects progressed from minimal intimal thickening and fraying of the internal elastic lamina of arteries in the 5 μc group (18,000-35,000 rads over a 36-45 month period) to narrowing or occlusion of the lumen by endothelial hyperplasia and fibrosis and eventual loss of nuclear staining in both the intima and media in the 240 μc/day group over a period of 11 months (greater than 100,000 rads). Ultimate complete destruction of vessels occurred with higher dosages.

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THE BLOOD PICTURE IN SUFFOLK SHEEP FOLLOWING SURGICAL THYROIDECTOMY

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

Surgical thyroidectomy increased blood creatinine, inorganic phosphorus, urea nitrogen, and neutrophils. Leukocyte and lymphocyte counts decreased. Cholesterol was found to be lower in the experimental animals than in the controls until 120 days after thyroidectomy, while calcium values were reduced after this period.

The purpose of this study was to ascertain the specific effect of surgical thyroidectomy on certain blood constituents in order to have a basis for a comparison with values obtained after ablation or damage to the thyroid by I^{131}.

METHODS

Eight purebred Suffolk ewe lambs were used. Three served as controls and five were surgically thyroidectomized at five months of age. During the course of the experiment two of the animals succumbed at 164 and 269 days after thyroidectomy. Residual thyroid tissue was found to be present in one of the thyroidectomized animals as evidenced by I^{131} uptake following administration of a tracer dose of this isotope. Data from this animal were discarded when found to be consistently similar to control rather than experimental values.

At approximately weekly intervals blood was taken by jugular puncture, using a 21-gauge needle and evacuated tubes. Heparin was used as an anticoagulant. The determination of calcium, inorganic phosphorus, cholesterol, "apparent" creatinine, protein-bound iodine (PBI) and packed-cell volume, and the enumeration of erythrocytes were performed by methods described

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previously (1). A solution of 0.05 per cent methyl violet in five per cent acetic acid was used as a diluting fluid for the enumeration of leukocytes and the differentiation of polymorphonuclear and mononuclear cells. Differential counts obtained with this method were found to be more consistent than those obtained using Wright's stain, although the composite averages for both methods were the same. Hemoglobin was determined by the alkaline hematin method (2), creatinine by the Miller method (3), and urea by the microdiffusion method of Conway (4). The determinations of both "apparent" and "specific" creatinine were carried out to ascertain if any difference in trends existed. While "apparent" creatinine values were always higher than "specific" creatinine values, the pattern after surgery was the same for the two methods.

RESULTS

The average hematological values are shown in Table 1. Immediately following surgery a decrease in PBI and an increase in creatinine occurred. Urea nitrogen was not determined before surgery but was found to be significantly higher in the experimental animals than in the controls throughout the period investigated (90 to 360 days after surgery).

Serum calcium values were lower in the experimental animals subsequent to 120 days after thyroidectomy while inorganic phosphorus levels became increasingly higher over the entire period. Serum cholesterol was significantly lower for 120 days after surgery and then rose to approximately the same level as the control values.

No significant differences between control and experimental animals were observed in packed-cell volumes or hemoglobin. The lymphocyte counts were found to gradually decrease for the first several months after

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TABLE 1

Means Values for Blood Constituents

Constituent	Control Group	Pre-thyroidectomy	Post-thyroidectomy	Mean Difference Between Control and Experimental Animals
PBI (μg/100 ml)	4.06	3.45	0.80	3.26 ± 0.38*
"Apparent" Creatinine (mg/100 ml)	1.20	1.46	1.78	0.58 ± 0.14
"Specific" Creatinine (mg/100 ml)	0.92	0.89	1.43	0.51 ± 0.10
Cholesterol (mg/100 ml)	59.0	52.6	41.5 (before 120 days) 62.0 (after 120 days)	15.5 ± 7.2 None
Calcium (mg/100 ml)	11.5		11.0 (before 120 days) 10.5 (after 120 days)	0.5 ± 0.4 1.0 ± 0.4
Inorganic Phosphorus (mg/100 ml)	6.15	6.29	8.30	2.15 ± 0.69
Urea Nitrogen (mg/100 ml)	20.9		26.7	5.8 ± 2.1
Leukocytes (cells/cmm)	10,700	8,200	6,600	4,100 ± 690
Lymphocytes (cells/cmm)	7,900	6,800	3,300	4,600 ± 820
Neutrophils (cells/cmm)	2,300	1,300	3,300	1,000 ± 740
Packed-cell volume (%)	40.2	39.1	40.3	None
Hemoglobin (g/100 ml)	13.0	12.9	12.6	None

* 99% Confidence Limits

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surgery and then level off for the remainder of the experiment while the neutrophil counts were significantly higher in the experimental animals. The depression in the lymphocyte count was partially compensated for by an increase in neutrophil counts so that no decrease in total leukocyte count was evident for about two months after thyroidectomy.

DISCUSSION

Results obtained in this experiment for PBI, creatinine, calcium, and inorganic phosphorus were comparable with those of a previous study (5) of the effect of surgical and radiation (I^{131}) thyroid ablation. Changes in values for cholesterol, lymphocytes, and neutrophils, which were questionable in the previous experiment, were found to be significant in this one. The depression in lymphocyte count was not expected following surgical thyroidectomy. This significant finding suggests that both thyroid destruction and radiation probably contributed to the lymphopenia previously observed (1) following administration of thyroid ablative doses of I^{131}. It is perhaps significant that no lymphopenia developed when the surgical thyroidectomy was incomplete. It may be assumed that if a lymphocyte depression occurs following I^{131} administration and the thyroid is only partially damaged that radiation *per se* is responsible.

ACKNOWLEDGMENTS

We wish to express our thanks to the Clinical Laboratory personnel for their technical assistance and to the Statistics Unit for their analysis of the data.

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BETA IRRADIATION OF SKIN
I. GROSS AND HISTOLOGIC LESIONS IN SHEEP

L. A. George, Jr., S. Marks*, E. Jane Coleman and L. K. Bustad

Observations made over a twelve-month period of the gross and histopathologic lesions resulting from exposure of localized areas of sheepskin to 2000, 4000 and 8000 rads of beta irradiation are described. Areas exposed to 2000 rads revealed a slight but permanent increase in pigmentation. Marked and permanent epithelial hyperplasia with complete atrophy of all dermal appendages, followed by their limited regeneration characterized areas exposed to 8000 rads. The 4000-rad areas showed an intermediate degree of alteration of the various epithelial structures.

The experiment described was designed to determine the approximate threshold dose of beta irradiation, delivered by a P^{32} source, which is injurious to the skin of sheep and to develop and describe the pathologic syndrome resulting from such irradiation. The present paper describes the gross and histopathologic features of the syndrome over an observation period of twelve months.

METHODS

Three adult purebred Suffolk ewes were used in this experiment. The method employed in irradiating the sheep was previously described (1). Essentially it consisted of exposing localized areas of skin along the dorsolateral surface of sheep to a beta radiation source consisting of a P^{32} plaque 3 inches in diameter and delivering a surface dose rate of about 6800 rads per hour.

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RESULTS AND DISCUSSION

Gross Findings

The gross pathologic manifestations resulting from beta radiation of sheepskin, the exposure levels employed, and the times at which the effects were first recognized and at which they disappeared are shown in Table 1.

Although definite darkening of the skin was visible in all the irradiated areas, the concentration and distribution of the accumulations of pigment responsible for this phenomena were unusual only in those areas exposed to 8000 rads. On close examination of these areas a scattered distribution of very dense pigmented spots, irregular in outline and averaging approximately 3 mm in greatest diameter, could be seen. In the lower level areas the pigmentation was lighter and had a uniform distribution. A variation in the degree of epilation and the density and texture of the regenerated wool was observed among the three animals in those areas exposed to 8000 rads. Epilation was complete in one animal and incomplete in the remaining two animals. The changes in the density and texture of the regenerated wool consisted of a decrease in the density of wool fibers (i.e., number of fibers) and an increase in the number of coarse wool fibers in the 8000-rad areas. There was considerable variation in the degree of these changes among the three animals. The regenerated wool fibers in those areas exposed to 8000 rads have never attained the full length of the normal wool fibers in the surrounding areas.

Microscopic Findings

Table 2 describes the histopathologic features observed during the twelve months following irradiation of the skin. Figures 1, 2 and 3 show the microscopic appearance of control and irradiated areas of skin.

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TABLE 1

Gross Pathologic Changes Following Beta Irradiation of Skin

	2000 rads	4000 rads	8000 rads
Swelling	None	Onset immediate, severe, and diffuse. Duration about four days.	Onset immediate, very severe, diffuse (extending 1 cm beyond the border of the irradiated area). Duration four days.
Redness	None	Obvious but not severe.	Severe initially. Duration about four days.
Sensitivity	Very slight	Onset immediate. Duration about four days.	Onset immediate and very severe. Duration about six days.
Pigmentation	Very slight, but persisted	First noted during second week, persisted.	Very dense black pigmentation in the irradiated area. First noted during second post-radiation week, persisted.
Wool Changes	No cessation of wool growth No wool loss	Slight cessation of wool growth during first six weeks followed by normal growth. No wool loss.	Wool loss occurred during second and third post-radiation week, variable in extent and completeness. No regrowth until after the fourth month The new wool fibers did not attain the full length of normal wool fibers.

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TABLE 2

Histopathologic Changes Following Beta Irradiation of Skin

	2000 rads	4000 rads	8000 rads
Epidermis	An increase in pigmentation began during fourth week and persisted.	Thickening began during the fourth week and persisted. An increase in pigmentation began during second week and persisted.	Thickening began during third week and persisted. An increase in pigmentation began during second week, reached a maximum intensity by the fourth week and persisted.
Sebaceous glands	No change.	Began disappearing during fourth week. Disappearance was incomplete at eighth week when new glands began to reappear.	Definite reduction in size and number at fourth week and complete loss at ninth week. Normal glands began to reappear during twelfth week.
Apocrine glands*	Minimal degenerative changes during fourth to sixth week.	Incomplete loss at fourth week followed by a gradual return to normal number of glands.	Incomplete loss at ninth week. Return to the normal number of glands had not occurred at twelve months.
Hair follicles	No change.	Atrophy began during fourth week in many follicles. Normal follicles began to reappear during twelfth week.	Atrophy began during fourth week with maximum degeneration occurring at ninth week. First signs of regeneration were observed during twelfth week.

* Apocrine glands are superficially analogous in distribution and appearance to the sweat glands of certain other species.

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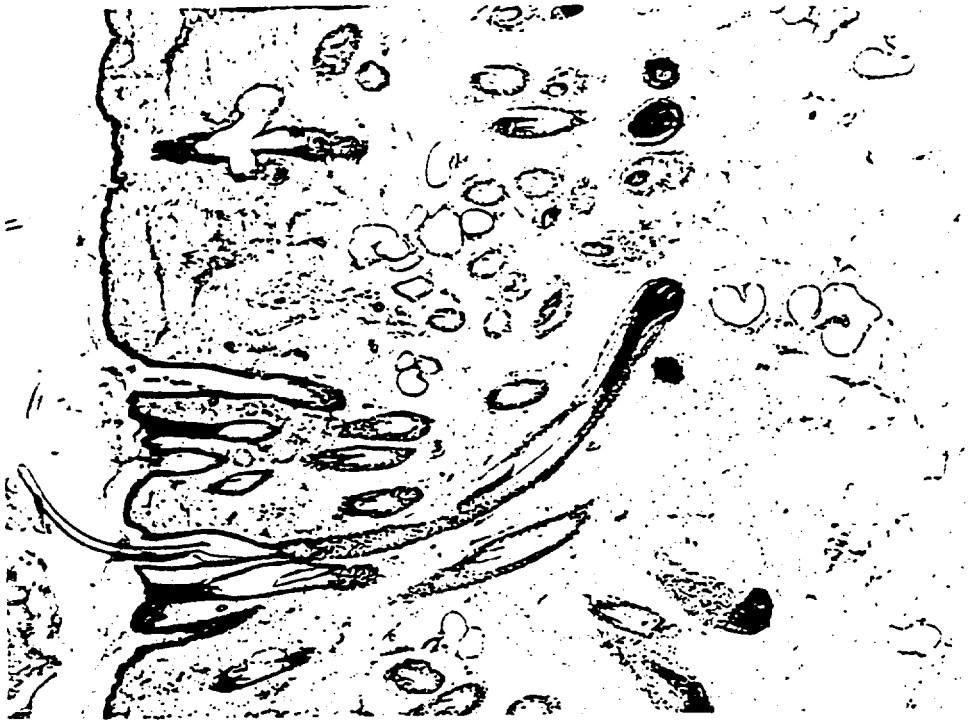

Irradiated Area

Control Area

Figure I

Photomicrographs of sheepskin showing the absence of structural alterations in the irradiated area four months after exposure to 2000-rad beta irradiation.
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Irradiated Area

Control Area

Figure 2

Photomicrographs of sheepskin showing the thickened epidermis and limited structural alterations of the dermal appendages in the irradiated area four months after exposure to 4000-rad beta irradiation. 54 X

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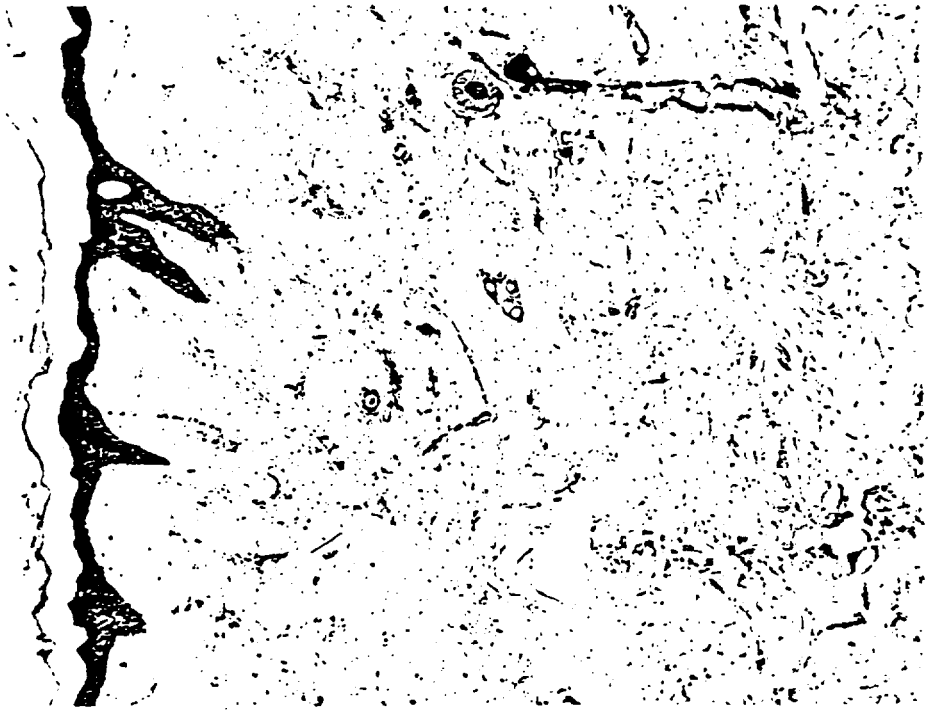
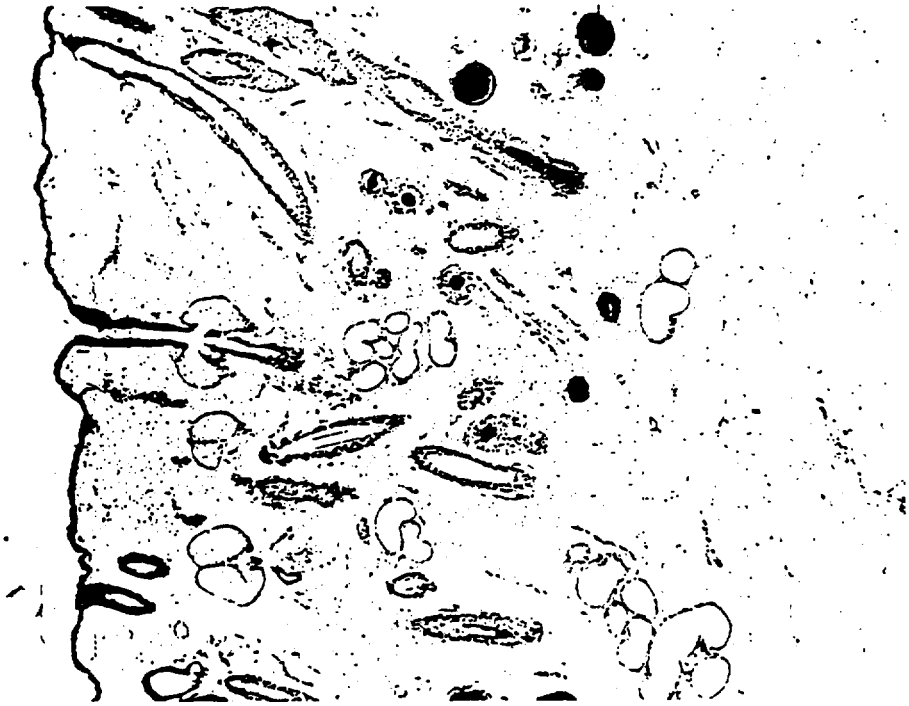


Irradiated Area



Control Area

Figure 3

Photomicrographs of sheepskin showing the thickened epidermis and severe alteration of dermal appendages in the irradiated area four months after exposure to 8000-rad beta irradiation. 54 X

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Epidermis

The thickened epidermis showed an increased number of mitotic figures in the stratum germinativium and, to a slight extent, in the deeper layers of the stratum spinosum.

The increase in the abundance and staining intensity of pigment granules was observed in both the epidermis and dermis. The pigment deposits in the epidermis were more prominent in the peripheral than the central portions of the irradiated areas. The distribution of pigment was focal throughout the thickness of the epidermis with the greatest concentration in the deeper layers.

Hair Follicles

The histologic effects in the hair follicles in those areas exposed to 4000 and 8000 rads were generally similar to the changes observed in the resting stage in the hair cycle of other species. These changes consisted of atrophy of the follicles with thinning of the epithelium of the root sheaths, serration of their margins, thickening of the glassy and connective tissue sheaths and a definite increase in pigmentation. The process of atrophy in the irradiated areas was imposed on most of the follicles within the area at one time and resulted in various degrees of epilation. These features are particularly significant in view of the fact that the follicles of sheep are normally in a continuous state of activity and do not exhibit the cyclic phenomena observed in other animals (2). In addition to the changes which are interpreted as potentially reversible, complete replacement of the bulbs of some follicles by scar tissue occurred, probably signifying permanent damage.

Glandular Appendages

Atrophy of both sebaceous and aprocrine glands occurred in the epilated areas (Figures 2 and 3). The apocrine glands are superficially

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analogous in distribution and appearance to the sweat glands of certain other species (3). The changes in these appendages may be attributed only in part to the direct action of radiation, since the sebaceous glands participate in the cyclic involution of the hair follicles and the apocrine glands may be responsive to effects on their excretory ducts. The relatively superficial sebaceous glands showed more severe atrophy than the deeper apocrine glands.

ACKNOWLEDGMENTS

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TOXICITY OF RADIOACTIVE PARTICLES
I. INTRATRACHEAL INJECTION OF RADIOACTIVE SUSPENSIONS

R. W. Wager and L. A. Temple

One hundred days after mice were injected intratracheally with plutonium oxide or plutonium hydroxide the incidence of pulmonary epithelial atypia was increased. Similar treatment with $Ru^{106}O_2$ caused increased incidence of pulmonary necrosis and fibrosis. Dibenz(a,h)anthracene increased the incidence of pulmonary necrosis, fibrosis, and papillary cystadenoma.

The purpose of this work was to estimate the tumorigenic effect of radioactive particles in the lungs of mice. At the same time, it was desired to investigate various techniques for intratracheal injection, crudely scope pulmonary turnover time and estimate deposition pattern obtained by intratracheal injection, and to determine resultant pathology. Both alpha and beta emitters were employed. The experimental period was arbitrarily limited to 100 days and very high doses were employed.

METHODS

Test Animals

Twelve-week-old BAF_1 female mice obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine, were employed in these experiments. The strain characteristically has a low incidence of pulmonary papillary cystadenoma which we considered desirable so that an increase in the tumor incidence due to treatments could be readily observed. Young mice were employed to avoid pathology appearing in older animals.

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Preparation of Particles

Plutonium oxide was prepared by heating triple-washed plutonium hydroxide in a muffle furnace at 700° C for two hours in a platinum boat coated with lucite (which facilitated subsequent removal of the PuO_2). The oxide was then ground under water and the resultant particles suitably classified as to size by sedimentation. Diameters of particles used ranged from 0.05 to 0.6 micron. Concentrations of plutonium oxide particles used were 2, 9, 20, and 25 μg/ml. A single concentration of 3 μg/ml plutonium hydroxide was prepared in which the mean particle size was 0.5 micron in diameter.

Ruthenium oxide was prepared by oxidation of the divalent metal to the gaseous tetroxide and subsequent reduction to the dioxide by alkaline ethanol. Subsequent treatment was similar to that used for preparing plutonium oxide particles. The mean particle diameter was 0.8 micron. The concentration of $Ru^{106}O_2$ used was 240 μc/ml.

Tween-80 was employed as a protective colloid in all final preparations.

Preparation and Injection of Mice

The animals were anesthetized with pentobarbital sodium. By blunt dissection, the trachea was exposed for a length of about 5 mm, and a dissecting needle was passed under the trachea to immobilize it.

Each suspension of particles was briefly exposed to ultrasonics to aid dispersion. Injections of 0.1 ml of the suspensions were accomplished by using a 25-gauge platinum needle attached to a fluorothene syringe fitted with a Teflon plunger. A glass syringe did not always permit quantitative delivery of PuO_2 due to adsorption on glass as noted by autoradiographs of lung slices.

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Pulmonary Distribution

From intratracheal injection experiments employing the dye, Cotton Blue, it was determined that non-uniform distribution of the radioactive particles could be expected to occur in the lung. However, when some degree of force was employed in injection, deeper penetration took place. The non-uniformity of distribution was verified from lung autoradiographs of mice injected with plutonium hydroxide.

Control Preparations

Positive and negative control preparations consisted of non-radioactive suspensions of dibenz(a, h)anthracene and barium sulfate A 1.0 mg/ml suspension of the chemical carcinogen was prepared by grinding the dibenzanthracene in an aqueous medium containing 0.5 per cent Tween-80. Mean particle size was 0.9 micron. Suspensions of barium sulfate for supplemental controls were prepared by grinding barium sulfate under water. For the higher concentration of barium sulfate, Tween-80 was added to 0.5 per cent. Other controls were solutions of sterile water and 0.5 per cent Tween-80.

Intravenous administrations of the chemical carcinogen and radioactive suspensions were performed in order to compare resultant pathology with that obtained from the pulmonary injections.

Analyses of Lungs

All animals were sacrificed by cervical dislocation. The ventrolateral portion of the thoracic cage was dissected away and the lungs were removed by gently lifting the free end of the trachea after it was severed at the laryngeal junction. The excised lungs were intratracheally injected with 80 per cent ethanol and the number of peripheral papillomae determined under a dissecting microscope.

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Measurement of plutonium in tissues was accomplished by digestion in hot, concentrated nitric acid to which was added, infrequently, small amounts of dilute HF to assure solution of PuO_2. Direct counts of alpha activity were made on evaporated aliquots.

Those tissues containing Ru^{106} were counted directly in the scintillation gamma counter.

All tissues submitted for autoradiographs were fixed in 80 per cent ethanol.

Induction of Lesions

Two types of lesions were noted: papillary cystadenomata and pneumonitis. Table 1 summarizes results obtained.

TABLE 1

Effect on Lungs of BAF_1 Mice 100 Days after I. T. Administration of Radioactive Suspensions

Material Injected	No. of Mice	Dose	% Mice w/ Tumor	Avg Tumors per Animal	% Mice w/ Pneumonitis
None	50	--	14	0.16	0
Dibenz(a, h)-anthracene	25	0.1 mg	68	2.5	16
Distilled water	25	0.1 ml	16	0.20	0
0.5% Tween-80	25	0.1 ml	8	0.08	0
$BaSO_4$ (in water)	50	3.0 μg	36	0.54	0
$BaSO_4$ (Tween-80)	55	5.0 μg	16	0.20	5
PuO_2	49	0.2 μg	22	0.24	8
$Pu(OH)_4$*	5	0.3 μg	20	0.40	40
PuO_2	25	0.9 μg	20	0.52	72
PuO_2	25	2.0 μg	36	0.60	28
PuO_2	41	2.5 μg	0	0.0	82
RuO_2	18	24.0 μc	0	0.0	61

* Assumed formula.

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An increased incidence of cystadenoma was observed only in those mice treated with dibenzanthracene while pneumonitis was noted in the animals treated with both the chemical carcinogen and the radioisotopes. Although there was no direct relation between the applied dose of the radioelement and the occurrence of pneumonitis, there is no doubt that pneumonitis was significantly increased by the applied radioisotopes.

The innocuous nature of barium sulfate has been noted previously by Huston (1) and Kay (2). However, we found that high doses of barium sulfate (5 μg) suspended in 0.5 per cent Tween-80 suggested increased pneumonitis. Yet when the barium sulfate was suspended in 0.1 per cent Tween-80 no increased incidence of pneumonitis was noted. An increase in papillary cystadenoma in mice due to atmospheric dust has been reported by McDonald (3).

Due to the 100-day limit of the experiment, one is unable unequivocally to state that radioactive particles do not increase the incidence of pulmonary cystadenomata in the mouse. The high dosages of radioactive materials applied to the lungs may have radiologically destroyed or modified cell populations destined otherwise to appear as cystadenomata. It was evident that the doses employed did result in inflammation of pulmonary tissue. That the destruction of pre-cancerous cells may have occurred is suggested by the fact that of the 18 animals which received 24 μc of $Ru^{106}O_2$ intratracheally, 7 were dead 36 days after injection, while 9 died between 36 and 71 days after treatment, and only 2 animals survived for 100 days after injection.

It is interesting to compare our high-dose results, both intratracheally and intravenously, with results obtained by Lorenz (4). After exposure to 8 r/day for a total of about 2500 r from an external source of gamma radiation, the lungs of tumor-sensitive mice exhibited a significant increase in papillary cystadenoma. No other pulmonary effects were noted

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In our work neither of the two surviving animals that received the $Ru^{106}O_2$ contained more cystadenoma than untreated controls. However, 24 animals were each injected intravenously with nearly 50 μc of radioactive ruthenium and after 100 days there was a significant increase in the incidence of cystadenoma. (The intravenous injection of the plutonium compounds had no noticeable effect on the lungs.)

These several results suggest that the intense radiation resulting from intratracheal injection of radioactive particles inhibits cystadenoma development while intravenously injected beta-gamma emitters accelerate it.

Description of Lesions

The papillary cystadenoma is a well-described lesion, readily recognized, occurring within the pulmonary parenchyma of certain strains of mice. The lesion is not malignant and is not believed to originate from the bronchial epithelium (5).

The histopathology caused by dibenzanthracene indicated necrosis as well as both deep and peripheral papillary cystadenomata. There was no obvious relation between the necrosis and the cystadenoma.

The pneumonitis resulting 100 days after intratracheal application of plutonium gave the lungs a hypochromic appearance, with leucomottling within the parenchyma due to necrotic areas. Histopathology associated with such areas is illustrated in Figure 1. Foci of bronchial epithelial proliferation frequently containing areas of metaplastic, stratified, squamous epithelium were noted.

The pneumonitis of ruthenium-treated lungs appears as a result of hemorrhage wherein the color of the tissue is dark red rather than the brilliance of fresh exudate. In Figure 2, a section from a lung indicates

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Figure 1
Section of mouse lung 100 days after intratracheal injection of 2.5 ug PuO_2 showing confluent areas of bronchial epithelial proliferation and metaplastic stratified squamous epithelium. 115 X

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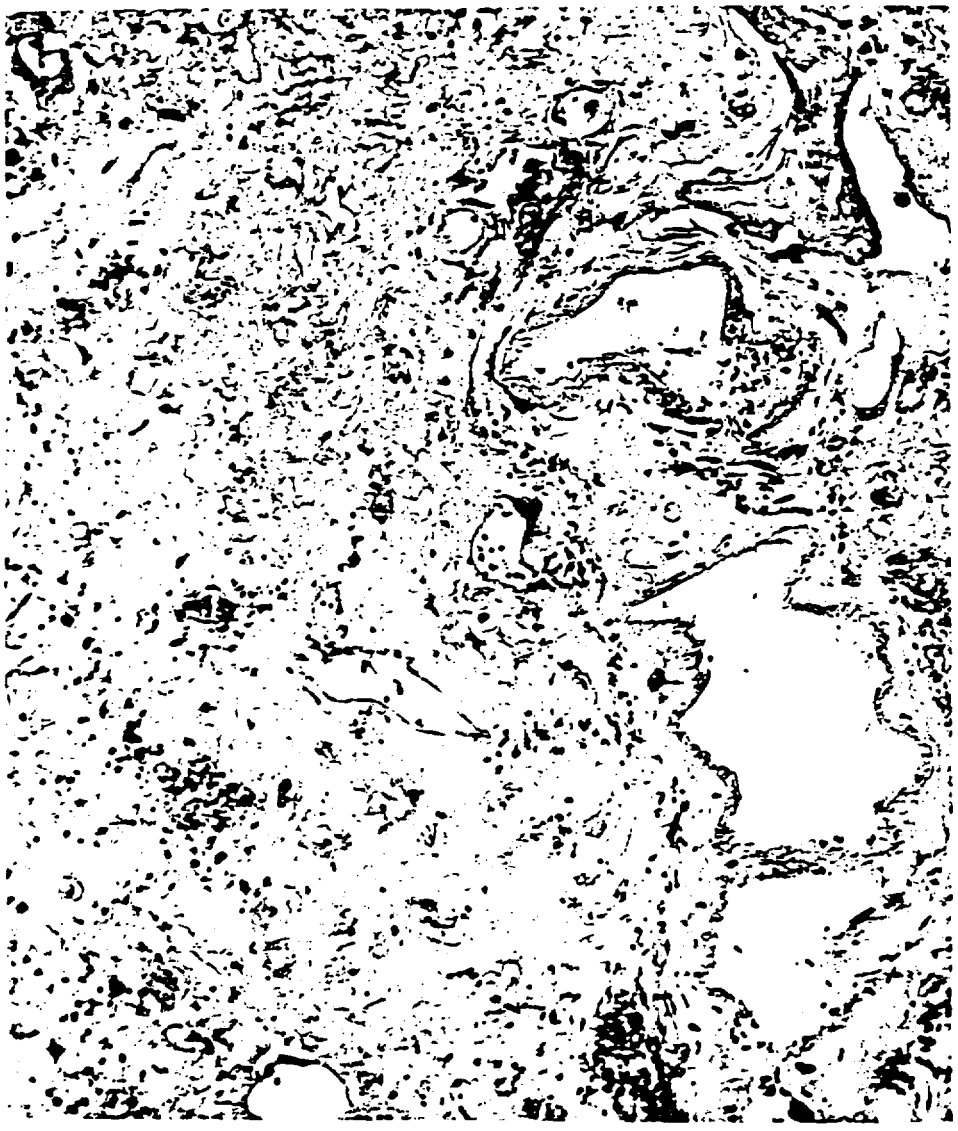

Figure 2
Section of mouse lung 100 days after intratracheal injection of 24 uc RuO_2 showing large fibrotic area containing cells with bizarre hyperchromatic nuclei.
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an absence of epithelial proliferation, but areas of necrosis and dense fibrosis containing large, bizarre, hyperchromic nuclei are apparent. In all cases pathology was accompanied by radioactive particles as shown by autoradiography.

It was interesting to observe the action of phagocytes as indicated by autoradiography. Fifteen minutes after intratracheal injection of a $Ru^{106}O_2$ suspension, particles were seen free in both bronchioles and alveoli, while eight hours after injection all the particles appeared within phagocytes. One hundred days after injection of plutonium oxide, particles were observed within phagocytes present in the lumen of the alveolar sacs (Figure 3).

Pulmonary Transport of Particles

The quantitative data on pulmonary particle removal rates are crude because the amount of plutonium administered was not accurately known. Furthermore, the effects of the large dose, non-uniform distribution, and pneumonitis, may have seriously altered physiological mechanisms devoted to pulmonary clearance of deposited foreign particulates. However, rough estimations based on the amounts of plutonium administered and on the amounts found in the lungs at different time intervals up to 100 days after injection indicated "biological half-lives" from 10 to 630 days.

ACKNOWLEDGMENTS

The valuable assistance of N. L. Dockum and E. Jane Coleman for the histological and autoradiographic preparations, and C. H. Hemphill for the preparation of samples for radioassay is gratefully acknowledged.

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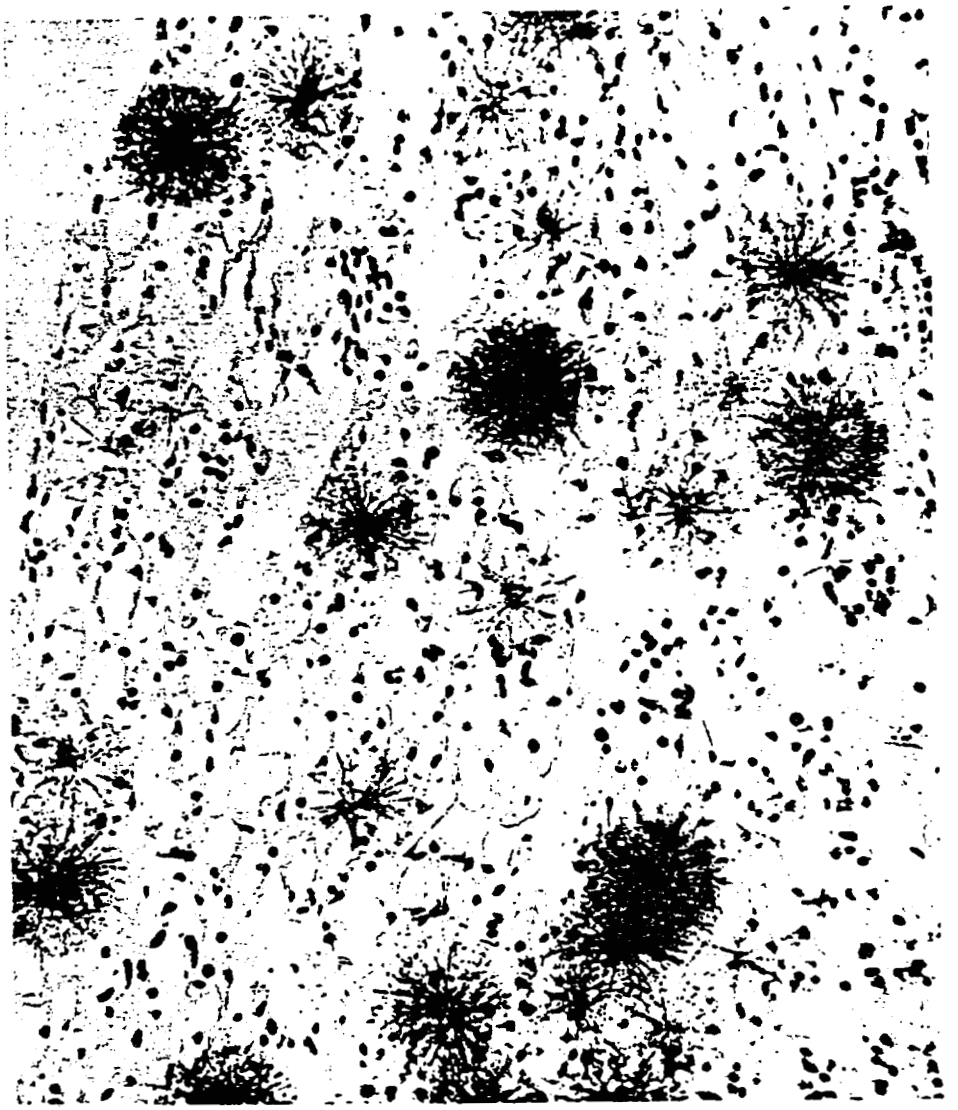

Figure 3

Autoradiograph of section of mouse lung one hundred days after I. T. injection 0.2 ug PuO_2. Note phagocytes containing PuO_2 and fine granules suggestive of hemosiderin. These phagocytes appear to have wandered from a concentration of particles not showing in this photograph. 125 X

AEC-GE RICHLAND WASH

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RELATIVE BIOLOGICAL EFFECTIVENESS OF IONIZING RADIATIONS BY MICROBIOLOGICAL METHODS

H. J. Dishburger, M. P. Fujihara, F. P. Hungate, and H. A. Kornberg

An improved procedure is described for determining the RBE of ionizing radiations on microorganisms. Results indicate that the RBE of tritium as compared with P^{32} is 2.0 or slightly higher for *L. casei* and yeast.

The microbiological method described for measuring the relative biological effectiveness (RBE) of ionizing radiations on bacteria (1) has been modified and extended to cover studies with yeast. Basically, the method is one of comparing the inhibition of growth during irradiation from a "standard" source of radiation with that from a source yielding particles of another type or energy Quantities of energy delivered by the standard and the other source are evaluated with a chemical dosimeter.

MATERIALS AND METHODS

The organisms used in these studies were a haploid and diploid strain of *Saccharomyces cerevisiae** and a strain of *Lactobacillus casei*. The yeasts were grown at 20° C in a modified Burkholder's medium (2), containing 0.1 per cent sugar, while the *L. casei* was grown in a minimal medium (3) at 30° C. The organisms were grown at the suboptimal temperatures in order to prolong theradiation delivered to the

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* Yeast strains were obtained from Dr. Hershel Roman of the University of Washington. The diploid (260-I) and haploid (27S-IX) strains are genetically non-flocculating sister clones.

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organisms between cell divisions, thereby increasing the test's sensitivity. Media were prepared at twice the final concentrations, sterilized and inoculated from a standard inoculum. To each tube 2.5 ml of medium were added aseptically, and isotope plus sterile water added to make a total of 5.0 ml. Tubes were generally set up in triplicate for each isotope and for the controls. Growth, as indicated by turbidity, was automatically recorded by an autoturbidimeter (4). The amounts of the two isotopes added to the solution were adjusted to give maximum probability of obtaining coincident growth curves.

The physical dose delivered to the cultures was measured by means of a chemical dosimeter (5, 6) utilizing the ferrous-ferric system in test tubes identical to those used in obtaining growth-time curves. Since pyrex tubes do not efficiently transmit light at the wave length required to measure the ferric ion, aliquots were transferred into 1 cm quartz cuvettes at the end of each experiment, and the relative amounts of ferric ions formed were measured directly at 305 mμ on a Beckman spectrophotometer. The quantity of ferrous ions oxidized to the ferric state and corrected for differences in ionic yield is taken as a measure of the physical doses delivered by the isotopes. Ionic yields for the ferrous-ferric dosimeter of 12.7 and 15.4 ions/100 ev for tritium and P^{32}, respectively, were used.

The beta particles from P^{32} were chosen as a standard source in these comparative studies, arbitrarily giving them the biological effectiveness of unity. The relationships used to derive a value for the relative biological effectiveness are as follows. For radiations from isotope #1 the biological response (R_1) is proportional to the energy available from the isotope $(mc \cdot E)_1$ times the fraction of energy absorbed by the culture (f_1) times the biological effect of the beta particles $(BE)_1$. This can be

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expressed as

$$R_1 = k(BE)_1 f_1(mc \cdot E)_1$$

and similarly for isotope #2

$$R_2 = k(BE)_2 f_2(mc \cdot E)_2 .$$

Since coincident growth curves were chosen, $R_1 = R_2$, and the ratio of the two biological effects is equal to the ratio of the total energies absorbed by the media in tubes containing the two isotopes.

$$\frac{(BE)_1}{(BE)_2} = \frac{f_2(mc \cdot E)_2}{f_1(mc \cdot E)_1}$$

Likewise the physical dose (D_1) is described as the energy available from the isotope $(mc \cdot E)_1$ times the fraction of energy absorbed by the equivalent culture volume (f_1) times the efficiency with which the energy is used to oxidize ferrous to ferric ions (G_1). This is expressed as follows for isotopes #1 and #2.

$$D_1 = f_1 G_1 (mc \cdot E)_1$$
$$D_2 = f_2 G_2 (mc \cdot E)_2$$

A combination of the biological and physical relationships under similar conditions results in a ratio of the biological effects which is equal to the ratio of the ferric ions formed times the ionic yield.

$$\frac{(BE)_1}{(BE)_2} = \frac{f_2(mc \cdot E)_2}{f_1(mc \cdot E)_1} = \frac{D_2 G_1}{D_1 G_2} = RBE$$

Thus the ratio of the energies delivered by the two isotopes, at concentrations that cause similar growth inhibition, becomes equal to the relative biological effectivess.

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RESULTS

Figure 1 illustrates typical experimentally determined growth-time curves showing the growth inhibition exhibited by the cultures in various concentrations of tritium and P^{32}. Optical densities of the cultures are plotted as a function of time. The points indicate the average value of the experimental findings. Values for the RBE of tritium as compared to P^{32} were found to be 2.0 with L. casei, and 1.8 and 2.3 when the diploid and haploid strains of S. cerevisiae were used as the test organisms. The values for yeast are tentative, being derived from only one test.

DISCUSSION

Growth responses in L. casei and S. cerevisiae were similar except that greater doses were needed to produce equivalent depressions of growth in yeast. RBE values were found to be relatively constant for various doses in the range of 8 to 45 thousand rads for L. casei and 20 to 100 thousand rads for yeast.

The RBE value of 2.0 for the effect of tritium beta particles on L. casei is somewhat greater than the 1.7 previously reported by Getzendaner, Fujihara, and Kornberg (3). This difference stems primarily from the difference in method of evaluating the dose delivered. Previously this was done by using small cuvettes for the chemical dosimetry and then mathematically correcting (7) for the difference in geometry between the cuvettes and the test tubes used for the cultures. The dose derived in this way for P^{32} is lower than the dose obtained by making the dose measurement in a tube identical with that used in the biological test.

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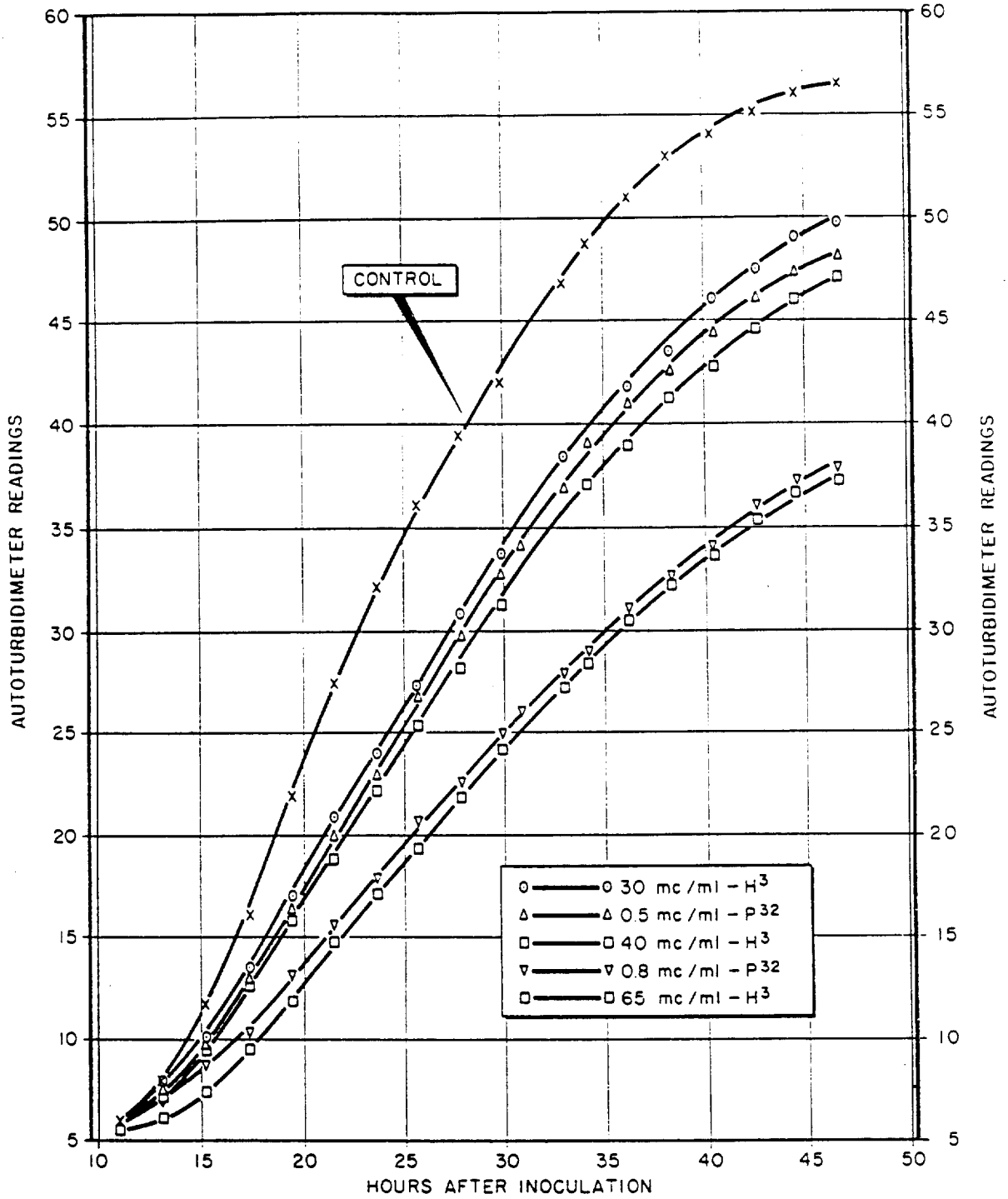

FIGURE I
TYPICAL GROWTH CURVE FOR L. CASEI
INHIBITION BY RADIATION FROM H^3 AND P^{32}

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ASSAY OF Fe^{55} AND Fe^{59} IN BIOLOGICAL SAMPLES

J. H. Rediske, R. F. Palmer, and J. F. Cline

A method for determining quantities of Fe^{55} and Fe^{59} in a mixture was devised, employing the formation of the iron thiocyanate complex and its extraction by an iso-amyl alcohol and di-ethyl ether mixture. The activity of each isotope is determined by comparing the counts with and without an absorber in a windowless counter.

Of the radioactive isotopes of iron, Fe^{59} is perhaps the most commonly used in tracer experiments because of its ease of detection. However, certain inherent characteristics of Fe^{55}, especially the longer half-life and softer radiation, have a definite advantage over Fe^{59} in biological work. Because of the softer radiation, methods for detection of Fe^{55} in the past have involved either the relatively inefficient process of x-ray detection by a thin window G-M tube (1) or the tedious electro-deposition of an infinitely thin sample (2).

In addition to the use of these isotopes individually, it is often desirable to introduce both Fe^{55} and Fe^{59} as a dual tracer in an experiment and determine the activity of each independently. Methods previously used (1, 2) for the simultaneous assay of these two isotopes have not proven practical for the particular experiments being conducted at this laboratory.

METHOD

The stock solutions of Fe^{55} and Fe^{59} used were standard Oak Ridge catalogue items, checked for radiochemical purity by decay analysis. Two samples were prepared by mixing aliquots from the two stock solutions in the same proportions but to different final concentrations. Portions of these samples were mixed with dry plant matter,

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digested, according to the procedure of Piper (3), and diluted to a known volume. To 1 ml of this digest were added ferric ion and thiocyanate. The mixture was then extracted with di-ethyl ether and iso-amyl alcohol. A portion of the organic phase was plated and dried. The residue was first counted directly on a windowless counter to obtain the total Fe^{55}-Fe^{59} count and then counted through a 0.63 mg/cm^2 rubber hydrochloride absorber (Reed Laboratories, Inc., Akron, Ohio) to obtain the Fe^{59} count.

RESULTS AND DISCUSSION

The counts recorded through the absorber were due principally to the Fe^{59}. From counts made on each isotope, it was determined that the absorber used allowed 66 ± 2 per cent of the countable radiation from the Fe^{59} to be counted. Only 1.5 per cent of the count from Fe^{55} was recorded through the absorber. The activity of each isotope was determined as follows:

If the total observed counts/minute with no absorber are

$$T = c/m\ Fe^{59} + c/m\ Fe^{55}, \text{ and}$$

if the observed counts/minute through the absorber are

$$A = 0.66\ c/m\ Fe^{59} + 0.015\ c/m\ Fe^{55}, \text{ then}$$

$$c/m\ Fe^{55} = \frac{0.66T-A}{0.645}, \text{ and}$$

$$c/m\ Fe^{59} = \frac{A-0.015T}{0.645}.$$

Based on the average of several determinations, one sample gave an Fe^{55}/Fe^{59} ratio of 0.69 and the other sample, which was at a higher concentration but the same ratio, gave the same value. These compare well with the calculated value of 0.70.

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To further test the validity of this method, sample 2 was allowed to decay for 15 days and the ratio again determined. The ratio had now increased to an average value of 0.88. This compares well with the value of 0.87 derived from calculating the effect of 15 days' decay of Fe^{59}.

The procedure as outlined has given satisfactory results in many analyses of mixtures of these isotopes. As applied to a mixture, or to each isotope independently, the method is more rapid and sensitive than the previous time-consuming electroplating method or the use of complicated auxiliary equipment to separate the two isotopes. It has the further advantage that analyses for radioactive iron may be carried out on the same solutions used for the thiocyanate colorimetric analysis of total iron (4).

A more detailed description of this method has been published elsewhere (5).

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"ELECTRON STAINING" REACTIONS OF COLLAGEN

R. Borasky and B. Mastel

The "electron stains" uranyl acetate and plutonium nitrate were removed from collagen fibrils by treatment with a chelating agent.

The purpose of this paper is to summarize preliminary findings on the behavior of plutonium nitrate as an electron stain for collagen fibrils and to determine whether destaining can be effected by chelating agents.

METHODS

Collagen fibril dispersions were prepared from air dried purified cowhide corium cubes by comminution in a blender. The collagen was allowed to react with uranium by mixing 9.0 ml of the dispersion with 1.0 ml of 0.1 per cent uranyl acetate aqueous solution. Other samples of collagen were allowed to react with plutonium by mixing 1.0 ml of the dispersion with 1.0 ml of buffered (pH 3.5) plutonium(IV and VI) nitrate solution, containing about 75 μg of plutonium. To determine whether uranium and plutonium could be removed from the stained collagen fibrils by a chelating agent, the fibrils were dialyzed against 0.5 per cent disodium ethylenediamenetetraacetate dihydrate (Na_2EDTA) solution, followed by dialysis against distilled water to remove excess chelating agent. Buffer salts were removed from the plutonium-treated dispersions by dialysis against distilled water. Samples were examined and representative fields photographed in the electron microscope at magnifications of 6300X and 10,000X.

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RESULTS AND DISCUSSION

The electronoscopic images of collagen fibrils have low electron scattering power and consequently very poor contrast. This is illustrated in Figure 1. Figures 2 and 3 show collagen fibrils "stained" with uranyl acetate and plutonium nitrate, respectively. Not only is the contrast enhanced by the incorporation of the high electron scattering power elements but also the "stain" clarifies the striated structures of the fibrils. The 650 A periodicity of the uranium stained fibrils is normal for mammalian collagen (1, 2).

The plutonium-stained collagen fibrils are slightly swollen because of the acid staining solution despite the presence of buffer. This is demonstrated by the 600 A period spacings in plutonium-stained collagen, Figure 3.

Attempts to destain "electron-stained" collagen by washing in water or dilute acids were unsuccessful. Figures 4 and 5 illustrate the destaining effect of Na_2EDTA on uranium and plutonium-stained collagen, respectively. Contrast is poor and there are no clearly defined striations. This indicates that the high electron scattering elements, uranium and plutonium, are removed from the collagen fibrils by the chelating agent.

These observations illustrate that plutonium is an "electron" stain for collagen and can be removed by the chelating agent Na_2EDTA.

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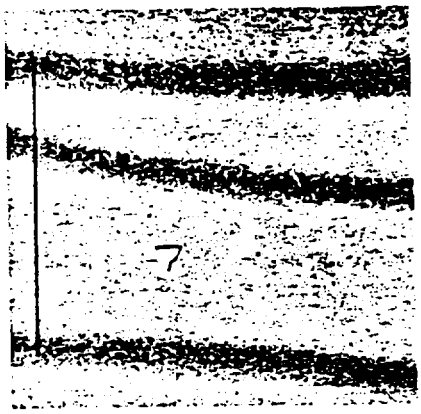

Figure 1

Cowhide Collagen Fibrils. 38,600 X
Note relatively poor contrast.

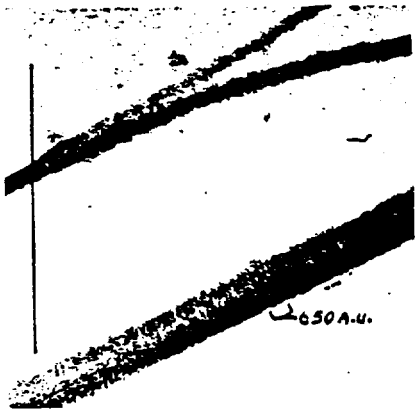

Figure 2

Cowhide Collagen Fibrils. 38,600 X
Stain, uranyl acetate.
Note 650 A period and 5 intra-period striations.

Figure 3

Cowhide Collagen Fibrils. 38,600 X
Stain, plutonium nitrate.
Note 600 A period and intra-period bands.

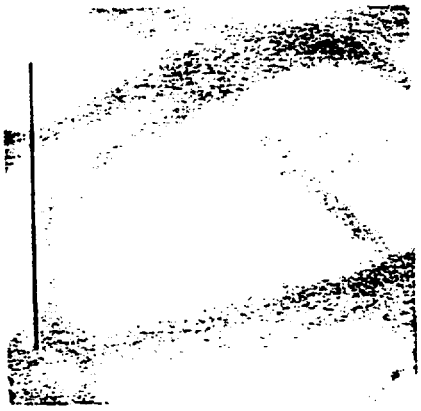

Figure 4

Cowhide Collagen Fibrils. 38,600 X
Stain, uranyl acetate; destained with Na_2EDTA.
Note absence of period and intra-period bands and poor contrast.

Figure 5

Cowhide Collagen Fibrils. 38,600 X
Stain, plutonium nitrate; destained with Na_2EDTA.
Note absence of period striations and poor contrast.

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EXTENSIBLE AREA SPECIMEN HOLDER FOR RCA-EMU MICROSCOPES

Rubin Borasky

A specimen holder was developed to increase the surveyable area of an electron microscope specimen grid from about 0.3 mm^2 to about 3.0 mm^2.

The maximum area of an electron microscope specimen grid that may be surveyed in an electron microscope is approximately 3.0 mm^2. When mounted in a conventional specimen holder and examined in an RCA-EMU microsocope equipped with a regular specimen stage, the area is restricted to the central 0.25 - 0.30 mm^2 of the grid.

The extensible area specimen holder described eliminates the necessity for handling specimen grids once mounted in the holder, and permits the examination of the entire area of the grid.

Basically the holder consists of two movable washers through which the cylindrical specimen holder passes. Screws adjust the position of the washers which move the specimen holder into the desired alignment. Figures 1A, 1B, 1C and 2 illustrate the component parts. Figure 3 illustrates how a specimen mounted out of range in a conventional holder is repositioned with the extensible area specimen holder.

This holder has been very useful in the examination of thin tissue sections, replicas of metal surfaces, and grids where the specimens of interest were distributed along the edges of the screen rather than in the center.

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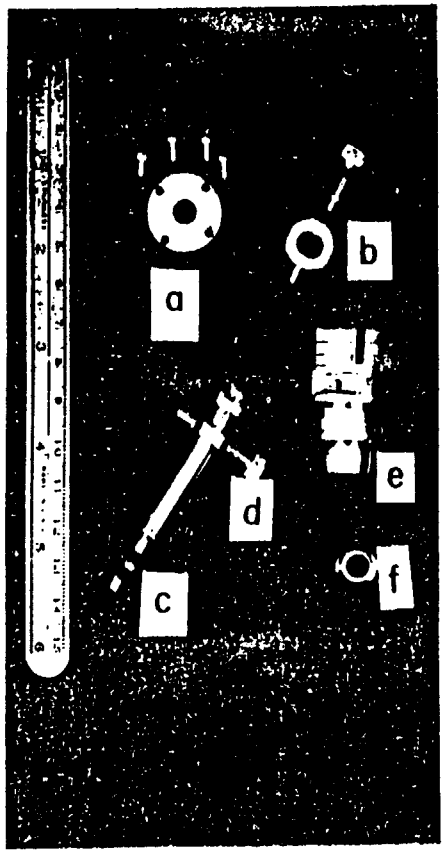

Figure 1 A

Component parts of the extensible area specimen holder.

(a) Retaining cap and screws; (b) upper member of mechanical stage, disassembled; (c) specimen grid and cap; (d) specimen holding component proper with lower member of mechanical stage; (e) collar and (f) retaining stabilizing washer.

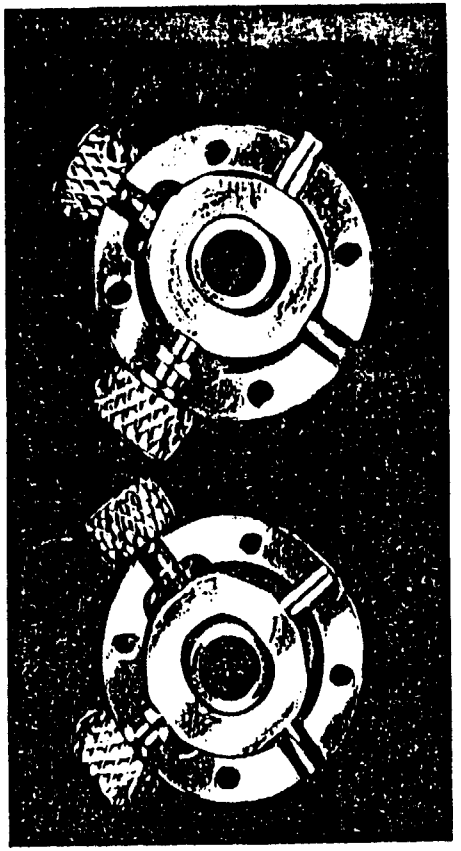

Figure 1 B

Top views of specimen holder, retaining cap removed.

Upper member of mechanical stage in its two extreme positions.

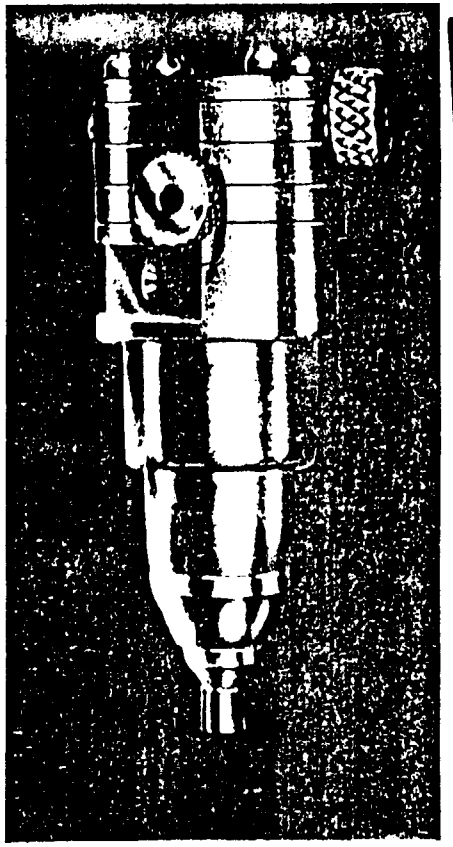

Figure 1 C

Extensible area specimen holder assembled.

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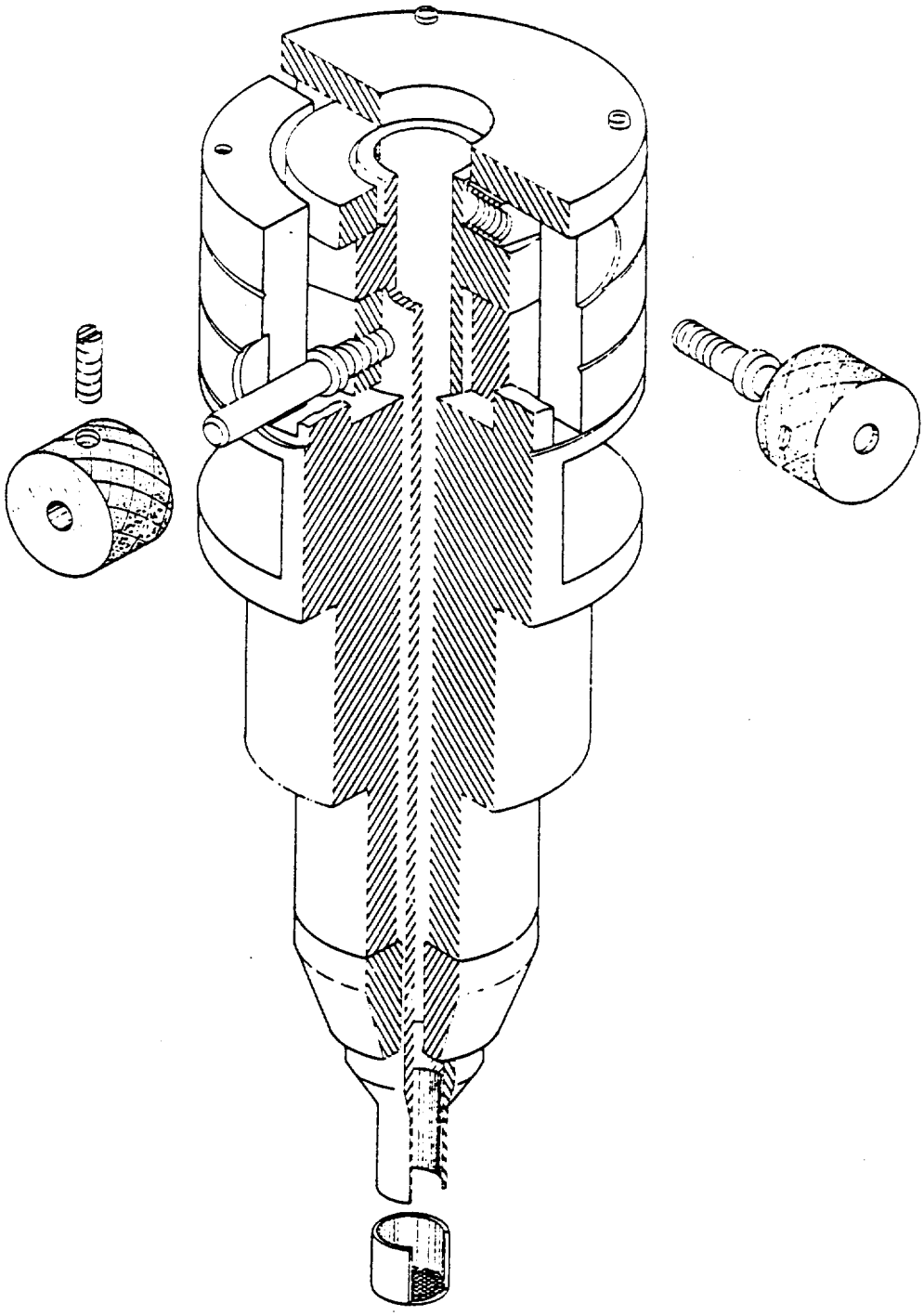

Figure 2

SECTIONAL VIEWS OF THE EXTENSIBLE AREA SPECIMEN HOLDER.

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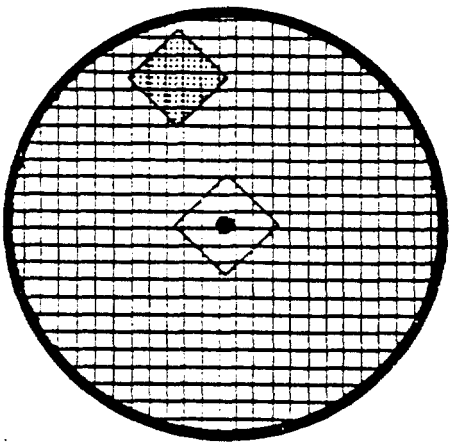

Figure 3 A

Specimen grid mounted in conventional specimen holder. Beam in center of grid with desired area (stippled) out of range.

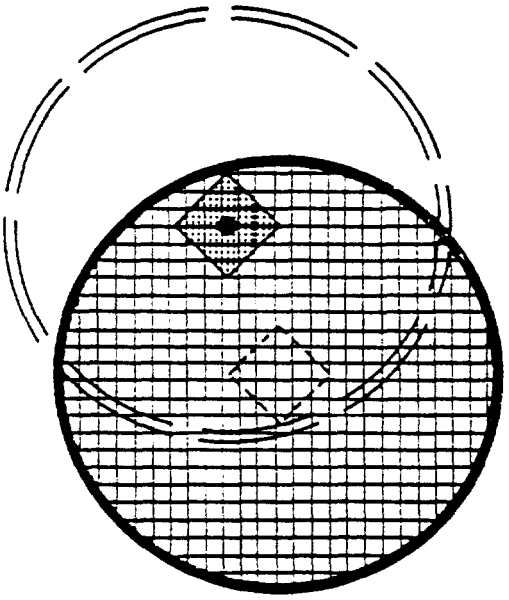

Figure 3 B

Specimen grid mounted in extensible area specimen holder. The screen is positioned so that desired area transects the electron beam.

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TALKS

Papers Presented at Meetings

Palmer, R. F., "Normal abundance of radium in cadavers from the Pacific Northwest," March 24, 1954, American Chemical Society Meeting, Kansas City, Missouri.

Thompson, R. C., "Gross metabolism of water in the rat as deduced from studies with tritium oxide," March 24, 1954, American Chemical Society Meeting, Kansas City, Missouri.

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Kornberg, H. A., "Relative biological effectiveness of ionizing radiations by a microbiological method," May 18, 1954, Radiation Research Society Meeting, Cleveland, Ohio.

Foster, R. F., "The physical and biological distribution of radioisotopes in the Columbia River below the Hanford reactors," May 25, 1954, Seventy-Fourth Annual Conference, American Water Works Association, Seattle, Washington.

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Bustad, L. K., "The care of sheep involved in radiation experiments," August 22, 1954, American Veterinary Medical Association Convention, Seattle, Washington.

Bustad, L. K., "Atomic bomb fall-out," August 22, 1954, American Veterinary Medical Association Convention, Seattle, Washington, (closed circuit television demonstration).

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Palmiter, C. C., "Effects of pile effluent upon Columbia River organisms," December 14, 1954, Omicron Society, Chico State College, Chico, California.

Pendleton, R. C., "Labeling plants and animals with radiosotopes for ecological studies," December 28, 1954, Ecological Society of America, A.A.A.S. Meetings, Berkeley, California.

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