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MEDICAL AND HEALTH PHYSICS QUARTERLY REPORT

January, February, March 1955

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MEDICAL AND HEALTH PHYSICS QUARTERLY REPORT

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MEDICAL AND HEALTH PHYSICS QUARTERLY REPORT

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STUDIES OF RADIOACTIVITY AND IRRADIATION

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TRACER STUDIES AND EFFECTS OF IONIZING RADIATION

THE DISTRIBUTION OF LARGE DOSES OF IODINE-131 IN THE RAT

R. W. E. Watts*

Hamilton, Durbin, and Parrott[1] estimated the MLD_{60} (Median Lethal Dose at 60 days) for I^{131} in rats, and reported the results of a limited histological study of tissues from the animals that had been given large doses of I^{131}. They concluded that although the acute mortality pattern following large doses of I^{131} was difficult to evaluate, the general findings resembled those following heavy whole-body x-irradiation.

The work reported here was designed to determine the localization of a sublethal, but radiotoxic, dose of I^{131} and, as far as possible, to compare this with the distribution of a small nonradiotoxic dose of the isotope. It was hoped that evidence would be obtained as to the tissues particularly exposed to radiation damage, and that further light would thus be shed on the cause of death following heavy internal irradiation with I^{131}.

Materials and Methods

Animals

Female Sprague-Dawley rats, 55 days old, averaging 150 grams in weight, were used. Water and the pelleted diet routinely in use at the Crocker Laboratory were given ad lib. After the administration of I^{131}, the animals were housed in groups of three per cage. The cages were shielded from one another by 2-in. thicknesses of lead and placed about 2 ft apart in order to minimize cross-irradiation between the animals in different cages.

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*Traveling Fellow of the British Postgraduate Medical Federation, University of London. Has been working with C. Willett Asling, Associate Professor of the University of California at Berkeley.

[1] J. G. Hamilton, P. W. Durbin, and M. W. Parrott, The 2nd Radioisotope Congress, Oxford, Proceedings Med. and Physiol. Applications 1, 219-231 (1954).

Experimental Procedures

All the injections were made into the surgically exposed external jugular vein under light ether anesthesia. Carrier-free I^{131} (obtained as NaI from the Oak Ridge National Laboratory) dissolved in isotonic NaCl was employed. The doses used were 30 μc/g body weight and 0.33 μc/g body weight, and the groups of animals were designated the high- and low-level-dose groups respectively. The animals were sacrificed, after the withdrawal of blood samples, at the following time intervals:

Time	Number sacrificed	
(Days after injection)	High-level-dose group	Low-level-dose group
7	5	5
14	6	5
21	7	5

After skinning, the animals were dissected, and the residue, "carcass," was passed through a meat grinder. The organs were weighed and their radioactivity measured with a scintillation gamma-ray counter. Where the counting rates obtained were too low for accurate measurement, the corresponding organs from two or more rats were pooled for assay. The results were expressed as (a) percent of the injected I^{131} present per whole organ and (b) percent of the injected I^{131} present per gram wet tissue; average values were calculated for each tissue at each time interval.

Results

Some degree of growth impairment was observed in the animals given the higher dose of I^{131}, but no animals died during the course of the experiment.

The detailed results of the average radioactivity measurements on the majority of the organs and tissues studied are presented in Tables I and II. In addition to the organs listed, the lacrimal, salivary, pituitary, and adrenal glands, lymph nodes, the thymus, the brain, and samples of adipose tissue were also investigated. In the low-level-dose group, these all contained 0.002 percent or less of the injected I^{131} per whole organ or tissue sample seven days after the injection, subsequently the amounts of I^{131} present were too small to be measured accurately, and the results on these tissues, together with the corresponding data from the high-level-dose group, have been omitted from these tables.

In general, the animals in the low-level-dose group retained a larger fraction of the injected material (expressed both as the percent per gram fresh tissue and as the percent present per whole organ). The spleen and pancreas were exceptional in this respect. The percentages of the injected I^{131} in the lymph nodes, thymus, ovary, lacrimal, and adrenal glands from the high-level-dose group of animals sacrificed at seven days were apparently greater than those in the corresponding tissues from the low-level-dose group. It is not, however, considered justifiable to analyze these exceptions further, because of the low counting rates involved in some cases.

Table I

The total amounts of I^{131} in the organs of rats 7, 14, and 21 days after intravenous injection. The animals in the high- and low-level-dose groups received 30 μc and 0.33 μc I^{131} per gram body weight respectively. The average values for each group are expressed as the percent of the injected dose present per whole organ or tissue mass x 10^3.

	7 days		14 days		21 days	
	Low-level	High-level	Low-level	High-level	Low-level	High-level
Bone*	57	23.3	35	14.1	12	16.1
Muscle**	207	132	157	59	36	39
Carcass	840	600	318	305	183	123
Pelt	1910	1390	1280	885	568	527
Thyroid	4010	51.4	2860	1.8	928	1.8
Heart	3	1.4	1	0.6	1	< 0.1
Lung	9	4.7	5	3.3	1	2.8
Spleen	1	1.0	1	0.6	1	0.5
Blood cells	17	3.0	12	1.1	---	1.7
Blood plasma	54	9.6	23	2.8	---	3.2
Liver	57	9.9	22	3.2	8	2.1
Kidney	13	3.5	5	1.8	2	1.6
Stomach	13	4.4	4	2.3	1	1.4
Stomach contents	55	13.1	23	5.5	2	4.6
Small intestines	27	8.8	9	3.3	4	3.1
S.I. contents	55	9.2	12	1.8	2	3.5
Large intestines	9	4.0	4	2.4	2	2.0
L.I. contents	88	7.8	21	6.0	4	1.5
Pancreas	4	2.3	3	1.4	9	1.4

*Skeleton assumed to account for 8% of the total body weight.
**Muscle assumed to account for 45% of the total body weight.

Table II

The concentration of I^{131} in the organs of rats 7, 14, and 21 days after intravenous injection. The animals in the high- and low-level-dose groups received 30 μc and 0.33 μc I^{131} per gram body weight respectively. The average values for each group are expressed as the percent of the injected dose present per gram of fresh tissue x 10^3.

	7 Days		14 Days		21 Days	
	Low-level	High-level	Low-level	High-level	Low-level	High-level
Bone	4	1.7	6	1	< 1	1
Muscle	3	1.7	2	0.7	< 1	0.5
Pelt	70	59	43	30	18	20
Thyroid	171,700	4,000	103,400	---*	35,000	175
Heart	4	2.1	1	0.9	< 1	0.4
Lung	8	3.8	4	2.6	< 1	0.8
Spleen	1	2.9	2	1.8	2	0.2
Blood cells	3	0.5	2	0.2	-	0.3
Blood plasma	8	1.3	3	0.4	-	0.4
Liver	7	1.2	2	0.4	< 1	0.3
Kidney	9	2.8	3	1.4	1	0.2
Stomach	15	4.7	33	2.3	2	0.4
Small intestine	6	2.2	2	0.8	< 1	0.3
Large intestine	5	2.4	2	1.3	< 1	0.3
Pancreas	< 1	1.4	< 1	0.8	4	0.1

*The thyroid glands in this group were very friable and for this reason they were not dissected off the adjacent portion of the trachea. The values for their weights therefore are not available.

Discussion

The widespread distribution of the injected isotope, with the retention of appreciable amounts in the pelt, muscles, skeleton, blood, and gastrointestinal tract, in these experiments indicates that a whole-body type of irradiation, as well as selective irradiation of the thyroid gland, occurs following the introduction of large amounts of I^{131}. This conclusion is in accord with the suggestion which Hamilton et al.[1] made on the basis on their morbid anatomical and histological studies. The relatively large amount of I^{131} present in the pelt may have been due to contamination with urine and saliva, so it is uncertain how large a contribution this would make to whole-body irradiation under conditions where such contamination did not occur. The presence of I^{131} in the skeleton would give rise to some degree of selective irradiation of the bone marrow, although in view of the decay scheme, relatively rapid elimination, and widespread distribution of the isotope, this would be, relatively, a less important hazard than with many other radioactive materials.

The retention of a larger fraction of the injected I^{131} per gram of tissue by some organs seven days after the injection in the animals given the larger dose of I^{131} may indicate that the size of the dose of I^{131} used influences in some way the concentration of I^{131} retained in these tissues. It is of interest that the spleen and possibly lymph nodes were among those showing this effect, and that it was only in the lymphoid tissues that Hamilton et al.[1] found histological evidence of radiation injury after the administration of 70 μc I^{131} per gram body weight. Such lymphoid tissue damage could contribute to death by impairing lymphocyte and antibody production.

When destructive amounts of I^{131} are taken up by the thyroid gland, the organic binding of I^{131} is abolished and the urinary excretion of the isotope in the form of iodide is therefore enhanced. This would explain the retention of a larger fraction of the injected dose of I^{131} by the animals given the smaller dose of I^{131}.

The nature of the I^{131}-containing compounds present in the tissues of animals in the high-level-dose group is not known. Tong, Taurog, and Chaikoff[2] obtained evidence for the presence of thyroglobulin in the blood plasma of rats 24 to 48 hours after the injection of large amounts of I^{131} (830 to 1000 μc rat). The fate of this iodoprotein when liberated into the blood stream under these conditions is not known. Thus, the I^{131} present in the tissues of the animals given 30 μc/g body weight could consist of one or more of the following: (a) iodide, (b) thyroid hormone produced between the time the gland began to take up I^{131} and the time it ceased functioning and (or) I^{131}-containing metabolites of the hormone, and (c) thyroglobulin and (or) its I^{131}-containing metabolites.

Summary

1. The distribution of I^{131} in the tissues of rats has been studied 7, 14, and 21 days after the intravenous injection of 30 μc I^{131}/g body weight which is approximately one third the MLD_{60}. Similar observations have been made after a relatively small dose of I^{131} (0.33 μc/g body weight), and the results obtained for the two groups have been compared.

[2] T. S. Tong, A. Taurog, and I. L. Chaikoff, J. Biol. Chem. 195, 407-413 (1952).

2. In general, both the percentages of the injected material present in the whole of each organ and the percentage present per unit weight of tissue were higher in the animals given the smaller dose of I^{131} than in the animals given the larger dose. A few exceptions to this generalization have been noted.

3. The significance of these findings is briefly discussed, with particular reference to the bearing on possible factors contributing to death following heavy internal irradiation with I^{131}.

Acknowledgments

The writer is pleased to record his indebtedness to Professor Joseph G. Hamilton and Professor C. Willett Asling for professional hospitality during part of the tenure of a traveling Fellowship awarded by the British Postgraduate Medical Federation, (University of London). The assistance of Dr. Patricia W. Durbin, Miss Margaret Gee, Mrs. Ruth Newman, Mrs. Marilyn Williams, and Miss Nylan Jeung is gratefully acknowledged.

ASTATINE STUDIES

THE PRESENT STATUS OF THE CROCKER LABORATORY MONKEY COLONY

Marshall W. Parrott

Two of the major problems in a monkey colony are tuberculosis and intestinal parasites, primarily worms. Both are of grave concern in every instance of the introduction of a new group of animals into an established colony.

New animals are immediately tuberculin-tested by injection of 0.1 ml of T.B., purified protein derivative, Parke-Davis and Company, intradermally in the upper lid of the right eye. This is repeated every six months in the established colony as well. Fortunately, these tests have yet to give a positive reaction in our colony. The worm problem, however, is another matter.

In the seven animals received in a shipment August 4, 1954, two types of worm eggs were observed by flotation of eggs in a hypertonic solution of $NaNO_2$. A fecal slurry in $NaNO_2$ is poured through a cheesecloth filter into a small vial until it is slightly overfull and surface tension keeps the liquid from running over the side. A clean slide is placed on this and left for a few minutes. The eggs float up to the slide and adhere to it. The slide is then inverted and cover-slipped. The various types of worm eggs may be seen under the low power of a microscope.

Various methods for removal of worms have been tried in the past with some success. A new vermifuge was tried but proved unsatisfactory in our colony, chiefly because of the size of the capsules and their undetermined result. We have since returned to hexylresorcinol, which has been fairly satisfactorily used in the past in the children's dosage in the form of "Crystoids" (small), manufactured by Sharp and Dohme Division of Merck and Co., Inc. Complete freedom of the colony animals from worms is important because the varities

of worms found are readily transmissible to humans, thus requiring diligent scrubbing each time one leaves the colony. The cages must also be thoroughly cleaned after each dose of "Crystoid", since the animals readily reinfect themselves.

Another condition provided a real problem and has remained unsolved to date. The outward physical appearance of a number of untreated control animals deteriorated over a period of about four months. These symptoms were accompanied by a severe anemia and a leucopenia. Lassitude replaced their normal curiosity, and eating habits changed markedly in the last stages of the illness, which was accompanied by almost complete constipation. This illness ultimately contributed to heart failure in two animals, and two more succumbed, probably to the same disease. The picture is somewhat obscure since certain aspects of the blood count resemble those of a worm-infested animal--strongylosis (hookworm) in particular. The diagnosis is difficult, for the slow decline in RBC count is hardly discernible from normal variation until a series of three counts, a month apart, is seen. Fortunately, a good record of blood counts has been kept on all the animals of the colony (with the exception of one, which is too difficult to handle except when the situation requires) since before the beginning of the most recent astatine experiment, begun in March 1953 (to be described later).

No trace of worms was found in the first two control animals that died February 7, 1955 (animals No. 6, "Smiley," and No. 3, "Woodie"). Bacterial cultures of the spleen and liver taken from "Woodie" proved negative on nutrient agar. Lung, spleen, liver, kidney, and adrenals of animals No. 3 and No. 6 were taken for histology. Dr. R. Lowry Dobson read these tissues in an attempt to determine the cause of death. The lungs showed macrophages from inhalation of small particles, possibly smoke. (Steps have been taken by Myron D. Thaxter to determine the source of the smoke and give some indication of possible elimination of smoke as a factor.) The lungs of one of the animals suggested a possibility of neoplasm. Liver sections appear slightly pigmented as though hemosiderin were present. The spleen had golden pigment usually present in hemolytic anemia. One of the animals had enlarged renal arteries. The adrenal glands were normal. Because of the severe terminal anemia seen in these two, the blood pictures of the remaining animals were checked. One animal, No. 16, was found to have this anemia but died during the night, autolysis making any histological investigation useless. On February 16 another animal, No. 15, began to show a much more rapid decline both in RBC count and general health, though the WBC's were not drastically affected. A pair of blood cultures, aerobic and anerobic, were run on No. 15 at Alta Bates Clinical Laboratory but have proved negative to this time. The animal was then found to be highly infested with strongyloid worms. Subsequent treatment with "Crystoids" has brought about rapid recovery.

On March 16 animal No. 5, "Squeaky," began showing the characteristic signs of the severe anemia about three weeks after the death of No. 3 and No. 6. Worm egg tests were attempted, but the infrequency and solidity of the stool samples made it impossible. Since the animal was one of a pair of males on a long-term astatine study, it was sacrificed and an autopsy was performed, because its death and the onset of autolysis at an undetermined time would seriously damage that experiment. Just prior to his death, 5 cc. of blood was removed and placed in two blood culture bottles which were taken to Alta Bates

Hospital for analysis of bacterial content. This analysis had not been completed at the time of this report.

In the hope of increasing the RBC count of those animals showing anemia, therapeutic doses of iron in the form of "Zymatinic" drops (vitamins B_1 and B_{12} and iron) were given on sugar cubes. Unfortunately, there was no immediate apparent effect, but the addition of further vitamins to the diet in the form of Vi-mix has resulted in some improvement in the entire colony.

The only other problem is that of colds and pneumonia. These animals are susceptible to practically all lung diseases. They pick up the colds of humans in the immediate area with no difficulty whatsoever. These may develop rapidly into pneumonia. Terramycin, aureomycin, or penicillin is used if the animals appear to be losing their normally sleek coat. Proper temperatures are extremely important to keep the animals from contracting any of the lung diseases. This is especially true of those animals that have received astatine in sufficient quantities to produce thyroid ablation. These animals lose their heat-regulating mechanisms; consequently, they must be kept even warmer than the 76° F required for the normal monkey.

The advice and assistance of Dr. Charles Riggs, D. V. M., Radiation Laboratory veterinarian, are gratefully acknowledged.

RETARDATION OF SKELETAL DEVELOPMENT IN MONKEYS TREATED WITH ASTATINE-211

C. Willett Asling and Marshall W. Parrott

A previous report (UCRL-2345, issued in April 1953) has dealt with the retarded skeletal development in a monkey in which cretinic dwarfism and myxedema had been induced by a thyroid-destroying dose of At^{211}. Since that time several immature monkeys, both male and female, have been subjected to this treatment, and among the other studies made, x-rays of representative parts of the skeleton have been taken at several intervals. This report summarizes the radiographic evidence for marked retardation of skeletal growth and maturation in these animals.

There were available for study x-rays of the shoulder, elbow, forepaw, knee, and foot of two male monkeys and two female monkeys that had been treated with astatine, and of two untreated male monkeys of about the same age. The age at the time astatine was injected was estimated on the basis of body weight and state of dentition; further age designations indicate the known period of observation of these animals.

Monkeys No. 1 and No. 4 were females, treated with 0.8 μc/g of At^{211} by intraperitoneal injection at the estimated age of 15 months. The first x-rays were made three months later, and subsequent observations were made 9.5 months after the injections. These animals are still under observation.

Monkeys No. 2 and No. 5 were males, treated with 0.36 μc/g of At^{211} by intraperitoneal injection at the estimated age of 12 months. The first x-rays were made three months later, and several observations were made over the ensuing 21 or 22 months.

Monkeys No. 3 and No. 6 were males, maintained as untreated controls. They were of the same estimated age as monkeys No. 2 and No. 5; the first x-rays were made at the estimated age of 15 months, and additional observations were made over the ensuing 10 months. A month ago they succumbed to an anemia and asthenia, the cause of which is under investigation; however, all of their x-rays were made while they were demonstrably in good health.

Growth

The third metacarpal bone and third metatarsal bone were selected for measurement on the basis that, since the paws could be flattened reasonably well, the x-ray measurements would be most likely be representative of the actual length of the bone without introducing substantial errors of projection enlargement or of foreshortening of the x-ray shadow due to angulation of the bone. Variability in measurement proved to be less than 1 part per 100 when the measurements were made with vernier calipers reading to 0.1 mm. This slight variability shows in the graphs (Fig. 1, length of third metacarpal, and Fig. 2, length of third metatarsal). It is obvious that none of the astatine-treated monkeys (open symbols, dotted lines) showed any growth of these two bones during the period studied. In contrast, the two controls (closed symbols, solid lines) showed a growth of 11% to 13.6% in the metacarpals, and of 7.4 to 9.4% in the metatarsals in the 9.5 months during which they were observed.

Structurally, the region of the epiphyseal plates, not only of the bones measured but of other bones as well, showed a sparsity and coarseness of the juxtacartilaginous bony trabeculae in the astatine-treated monkeys which was consistent with arrested endochondral osteogenesis; in later x-rays a transverse bony lamina appeared across the diaphyseal side of the epiphyseal plate, corresponding to the "line of arrested growth" described by H. A. Harris[1] and other workers. In contrast, in the controls there could clearly be seen a fine striated shadow in the juxtacartilaginous area representing primary bony trabeculae, with coarser secondary trabeculae appearing deeper in the shaft, a picture consistent with the active endochondral osteogenesis which produced the growth demonstrated in these controls.

Maturation

In all x-rays, even the earliest, there were already present the epiphyseal ossification centers of humerus, radius, ulna, distal end of femur, tibia and fibula, metacarpals, metatarsals, and phalanges, and the primary ossification centers of all carpal and tarsal bones. Judgments of any delay in skeletal maturation, therefore, must rest (1) on delay or failure to acquire the numerous secondary ossicles found in the paws of monkeys (sesamoid bones at metacarpo- and metatarsophalangeal joints, and certain carpal, popliteal, and tarsal ossicles), (2) on failure of the established epiphyseal ossification centers to expand normally, and (3) on failure of epiphyseal fusion to occur. This last was of very limited application, since the observations do not extend into the age group where normally the majority of long bones show epiphyseal union.

[1] H. A. Harris et al., "Bone Growth in Health and Disease", London, Oxford University Press, 1933.

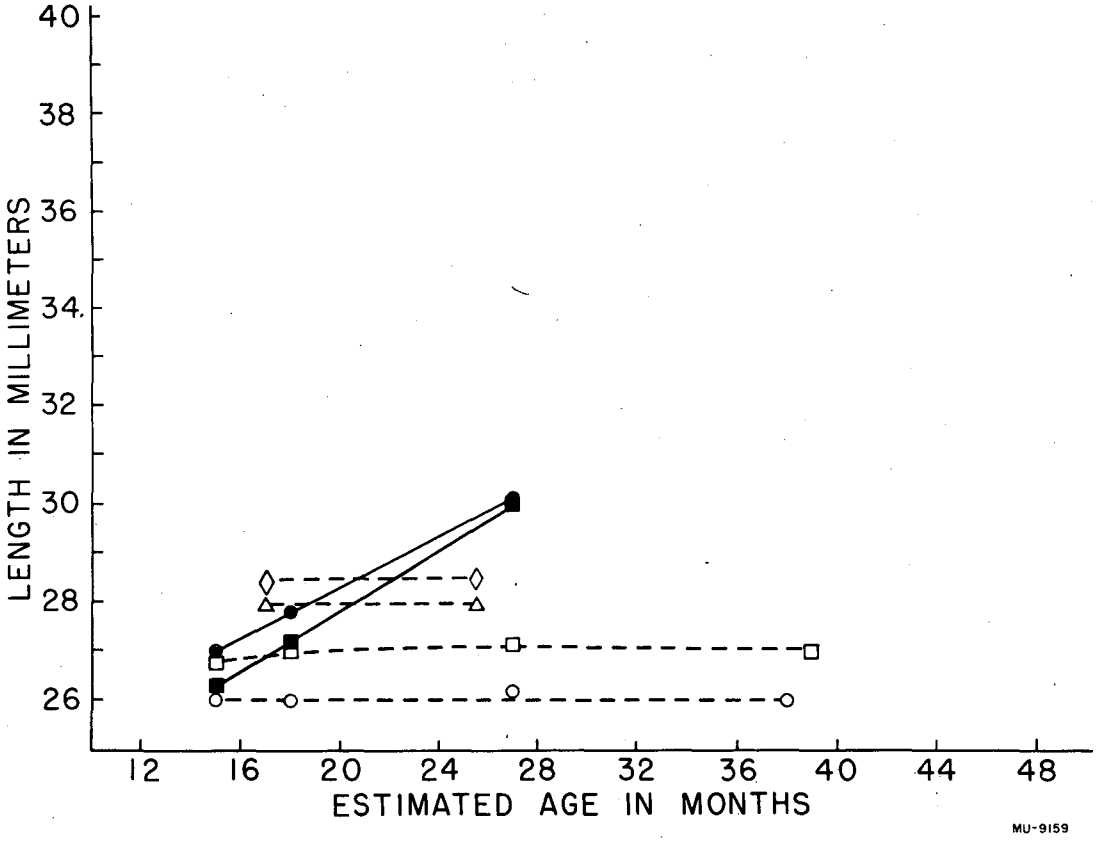

Fig. 1. Length of third metacarpal bone in astatine-treated monkeys. Control monkeys, closed figures, solid lines; At^{211} monkeys, open figures, broken lines.

◊ Monkey No. 4, female, 0.83 μc/g;
△ Monkey No. 1, female, 0.83 μc/g;
○ Monkey No. 2, male, 0.36 μc/g;
□ Monkey No. 5, 0.36 μc/g;
■ Monkey No. 3, male control;
● Monkey No. 6, male control.

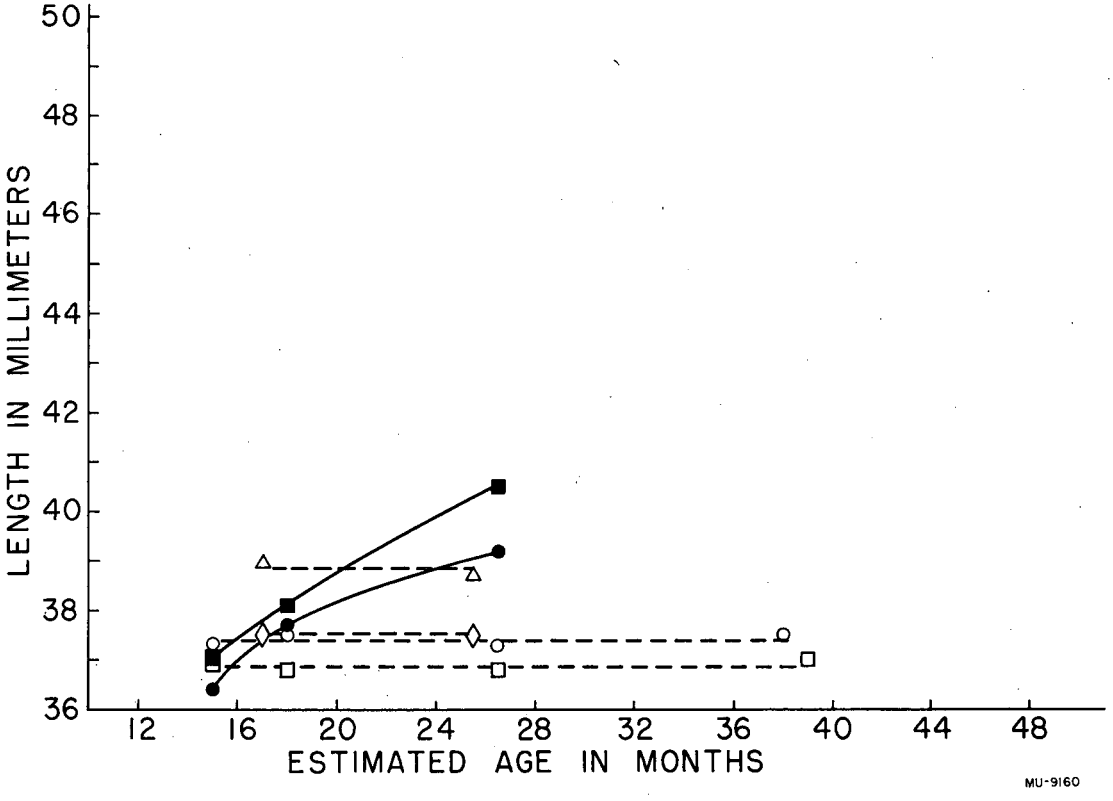

Fig. 2. Length of third metatarsal bone in astatine-treated monkeys. Control monkeys, closed figures, solid lines; At^{211} monkeys, open figures, broken lines.

◊ Monkey No. 4, female, 0.83 μc/g;
△ Monkey No. 1, female, 0.83 μc/g;
○ Monkey No. 2, male, 0.36 μc/g;
□ Monkey No. 5, 0.36 μc/g;
■ Monkey No. 3, male control;
● Monkey No. 6, male control

Monkey No. 1

Three months after astatine administration, when the animal was approximately 15 months old, the forepaw showed sesamoid ossicles at all metacarpophalangeal joints, and two small radial carpal sesamoids. In the hind paw the sesamoid ossicles were similarly all present, and a lateral tarsal sesamoid was visible. At the elbow, the olecranon epiphyseal center was already well expanded; all epiphyseal plates were open. At the knee, two ossicles were found in the popliteal region; a slight spike on the anterior aspect of the tibia near its epiphyseal plate represented the beginning of the tibial tuberosity. Nine and one-half months after the injection of At^{211} the radiographic appearance of all ossification sites was still just as described above. In particular, the carpal and tarsal sesamoids had not undergone any expansion, nor had the popliteal sesamoids nor the beginning tibial tuberosity. No epiphyseal unions had occurred.

Monkey No. 4

Three months after the At^{211} injection, when the animal was approximately 18 months old, the forepaw showed sesamoid bones under the first to the fourth metacarpals, but not under the fifth. There was one radial carpal sesamoid. The hind paw showed sesamoids under all metatarsals, and showed a lateral tarsal sesamoid. There were two ossicles in the popliteal area. The tibial tuberosity had not appeared. There was evidence of beginning epiphyseal union at the distal end of the humerus. Nine and one-half months after the injection of At^{211}, no changes were seen in the radiographic appearance. In particular, the sesamoid of the fifth digit of the forepaw had not appeared, the carpal and tarsal sesamoids and the popliteal sesamoids had not expanded, no beginnings of the tibial tuberosity were seen, and no further progress toward distal humeral epiphyseal union had been made.

Monkey No. 2

Three months after At^{211} injection, when the animal was approximately 15 months old, the forepaw showed sesamoids associated with the third and fourth digits, a questionable shadow for the second digit, but none for the thumb and the fifth digit. There were no carpal sesamoids. The hind paw showed sesamoids associated with the first four digits. There was no tarsal sesamoid. At the elbow the olecranon epiphyseal center was small. At the knee a small single popliteal sesamoid could be seen; the patellar center was small. Six months after At^{211} injection the only change was that the sesamoid for the second digit of the forepaw, questionable earlier, was definitely established. No other ossicles appeared, nor did those already present expand. In fact, some appeared slightly less dense. There was also an impression that the epiphyseal plates were somewhat narrower. No further change was observed in x-rays made 12.5 months and 24 months after At^{211} administration.

Monkey No. 5

Three months after At^{211} administration, when the animal was approximately 15 months old, the forepaw showed no sesamoids, either associated with digits or carpal bones. In the hind paw, the great toe possessed a sesamoid, as did the third and fourth digits; the second digit showed a questionable shadow,

and no ossicle was found associated with the fifth digit. There were no tarsal sesamoids. At the elbow the olecranon ossification center was notably small. At the knee the fibular epiphyseal center was also small; only one small popliteal sesamoid had developed. In all subsequent x-rays, covering a period up to approximately 37 months of age, there were no noticeable changes with respect to the establishment of new ossification centers or normal expansion of the existing ones. In this animal the lines of arrested growth were particularly conspicuous in an x-ray 12.5 months after At^{211} injection.

Monkey No. 3

In this control monkey the first x-ray, made at the estimated age of 15 months (a chronologic age approximating that of the first observation on the astatine-treated males No. 2 and No. 5), showed no sesamoids in the forepaw. They appeared (at 18 months of age) on the second, third, and fourth digits, and (at 24.5 months) on all forepaw digits and as a radial carpal sesamoid. In the hind paw sesamoids were found on the first to third toes and questionably on the fourth, at 15 months. This last was determinate at 18 months, and at 24.5 months the fifth digit also possessed its accessory ossicle. At the elbow the olecranon ossification center, originally small, underwent progressive expansion; the cartilage plate of the distal humeral epiphysis narrowed progressively. At the knee a questionable popliteal shadow at 15 months progressed to two well-developed ossicles at 18 months, which continued to expand subsequently. The patella underwent expansion over the observation period.

Monkey No. 6

At approximately 15 months of age the forepaw of this control showed sesamoids under the first four metacarpals, and the hind paw showed such bones under the first three metatarsals with a questionable shadow on the fourth. At 18 months of age a small radial carpal sesamoid had been added, and the hind paw showed a definite shadow for the fourth digital sesamoid. At 24.5 months all digits showed their sesamoids, the radial carpal sesamoid was large, and a well-developed lateral tarsal sesamoid was found. At the elbow the olecranon ossification center expanded during the observation period, and the distal humeral epiphyseal plate became narrower until at 24.5 months of age the bone showed partial epiphyseal union. The knee showed one popliteal ossicle at the earliest period, and three months later possessed two well-developed ossicles. The patella expanded progressively through the observation period.

Summary

Evidence has been presented that in four immature monkeys (two female and two male) treated with thyroid-damaging doses of At^{211}, there was neither skeletal growth nor maturation occurring during a period of observation commencing three months after the treatment and extending for as long as 24 months after the treatment. In contrast, two controls (male) of comparable age showed easily demonstrable growth and maturation. Absolute estimates of bone age have not been made in the absence of definitive x-ray bone-age tables for monkeys. An attempt to obtain such data is under way; further attempts at more precise estimates of the chronologic ages of these animals are also being made.

Hamilton

The skeletal retardation in the injected monkeys is consistent with the diagnosis of cretinism and myxedema, which diagnosis was based on the facies of the animals, and on tests for thyroid function.

THE PREPARATION OF ASTATINE-211

Marshall W. Parrott

During the past two years the production of At^{211} has been below the level required for large-scale animal studies. In an effort to increase the efficiency of the distilling procedure in microcuries of At^{211} per microampere-hour of bombardment, a close check was made on method versus yield. It was found, through changing only one variable at a time, that it was possible to substantially increase the yield. Some of these variables were the diameter of certain sections of the distilling apparatus, the rate of flow of streaming gas through the system, and distilling temperature. Increasing the temperature brought about considerably higher yields of At^{211} but introduced a colloid which resulted in errors in both assay and animal data. This problem was eliminated by ultracentrifugation of the activity prior to assay and injection. Further investigations have shown no colloidal material present after centrifugation. These and other minor changes in the method have resulted in the increased yields shown in Table III. The dimensions of the cyclotron beam seem to be a determining factor in the more recent yields. Variation in beam height or a change in the target position may eventually lead to yields far superior to those now obtained.

Table III

The yield of At^{211} from the 60-inch Cyclotron from September 1954 to March 1955. All yields corrected for decay to four hours after end of bombardment.

Date	Bombardment Time (μah)	Total Yield (mc)	μc/μah
9/14/54	7.5	0.250	33.4
9/21/54	4.0	0.096	23.8
10/6/54	20.7	1.400	67.8
10/12/54	94.0	6.850	73.1
11/4/54	40.5	2.000	50.0
11/11/54	9.92	0.390	39.5
12/14/54	50.3	3.360	67.5
12/21/54	20.0	2.040	102.0
1/26/55	50.0	4.069	81.3
3/2/55	69.0	4.970	72.0
3/16/55	55.0	6.800	117.2
3/22/55	90.2	11.630	130.0

AN IN VIVO COUNTING APPARATUS

George Barr, Margaret Gee, and Marilyn Williams

Introduction

The apparatus described below was designed for the periodic determination of I^{131} and At^{211} uptake in the thyroids of intact rats. Thyroid uptake is usually determined by in vitro methods, and because the animals must be killed, study of uptake rates in individual animals is usually impossible. Many animals are required, making a study expensive if special techniques have been used.

In other in vivo counters the animals have been held upside down, with the counting tube mounted over the thyroid region. Rats in this position struggle violently, and very severe restraint and (or) sedation is usually necessary. The counter described here permits placement of the rat in the holder in prone position with little restraint.

Design of Apparatus

A sodium iodide crystal, 1 in. diameter by 0.375 in. thick, was mounted above an RCA No. 6199 photomultiplier tube. The crystal was covered with a 200 mg/cm^2 aluminum filter. A cylindrical lead shield, 0.75 in. thick on top and 1 in. thick on the walls, surrounded the crystal with a 0.5-in.-diameter aperture directly above the crystal. This shield was adequate to reduce the I^{131} leakage to less than 1 percent. The scintillation unit was used with a Tracerlab autoscaler. For details see Figs. 3, 4, and 5.

Using the Apparatus

Because it seemed that sedation would be needed to keep the rats quiet in the holder, Serpasil (a sedative alkaloid) was tried. The in vitro 24-hour thyroid I^{131} uptakes of Serpasil-treated (3.0 mg/kilo) and normal rats were 14.4 percent and 13.3 percent respectively. The difference was not considered significant for the ten rats studied. The rats were lethargic and easily handled, but administration of Serpasil tends to induce diarrhea, and it was felt that the disturbance of the intestinal tract might invalidate excretion rate studies.[1]

A technique for using fully conscious rats in the holder was developed. This included placing each rat to be used in the holder once a day for two or three days prior to experimental use. Female Sprague-Dawley rats have been found tractable and easily trained. The entire process of mounting, counting, and releasing a rat can be accomplished in about three minutes. Minimal restraint is important, because there is then a minimum of defecation and urination while the animal is in the holder, and excretion rate studies are not disrupted. This is especially true for animals that have been trained in the holder.

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[1] R. W. Miner (Editor), Reserpine (Serpasil) and Other Alkaloids of Rauwolfia Serpentina: Chemistry, Pharmacology, and Clinical Applications. Ann. N. Y. Acad. Sciences 59, 1-140 (1954).

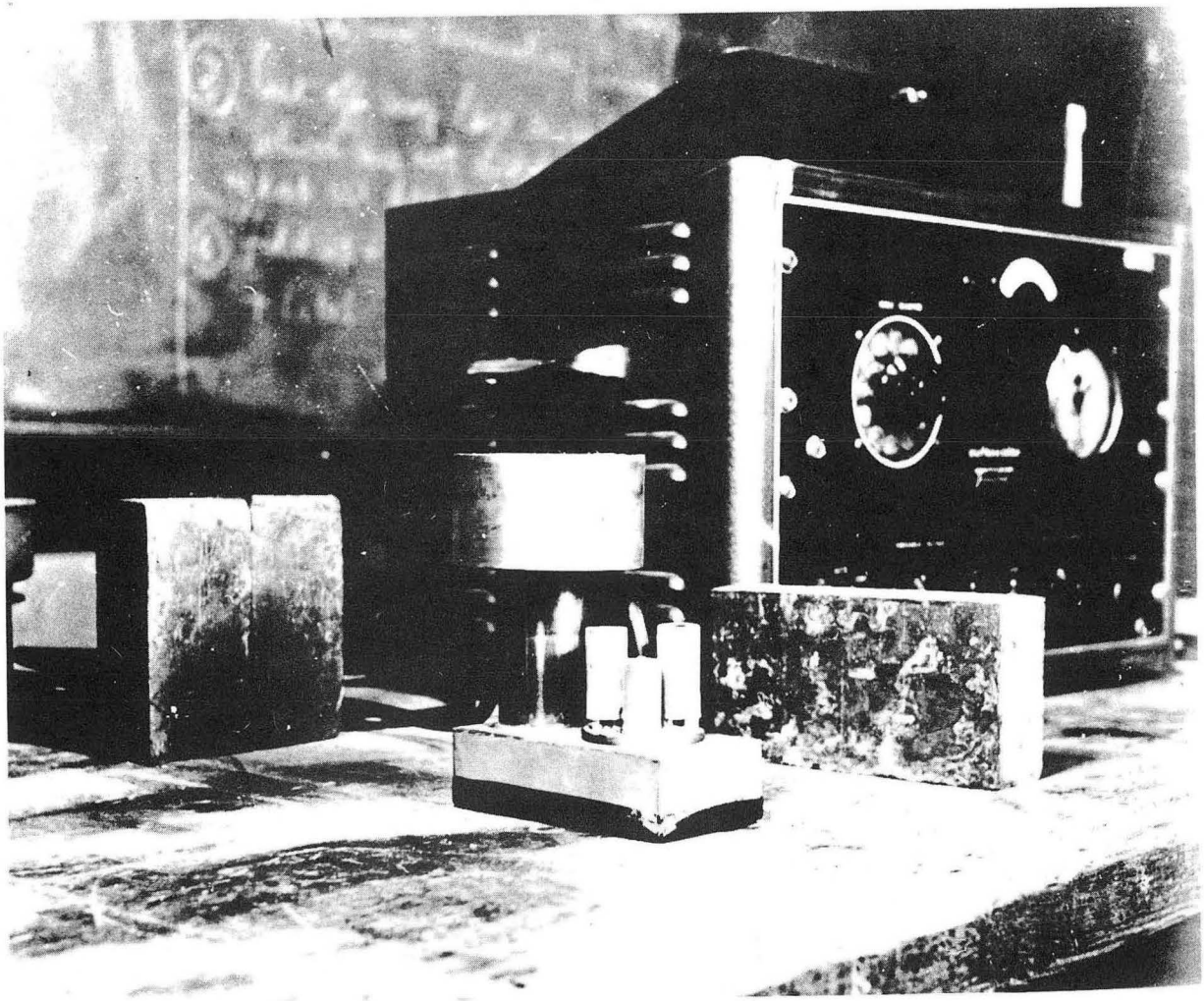

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Fig. 3. In vivo scintillation apparatus showing auto scaler scintillation unit and lead shield for crystal.

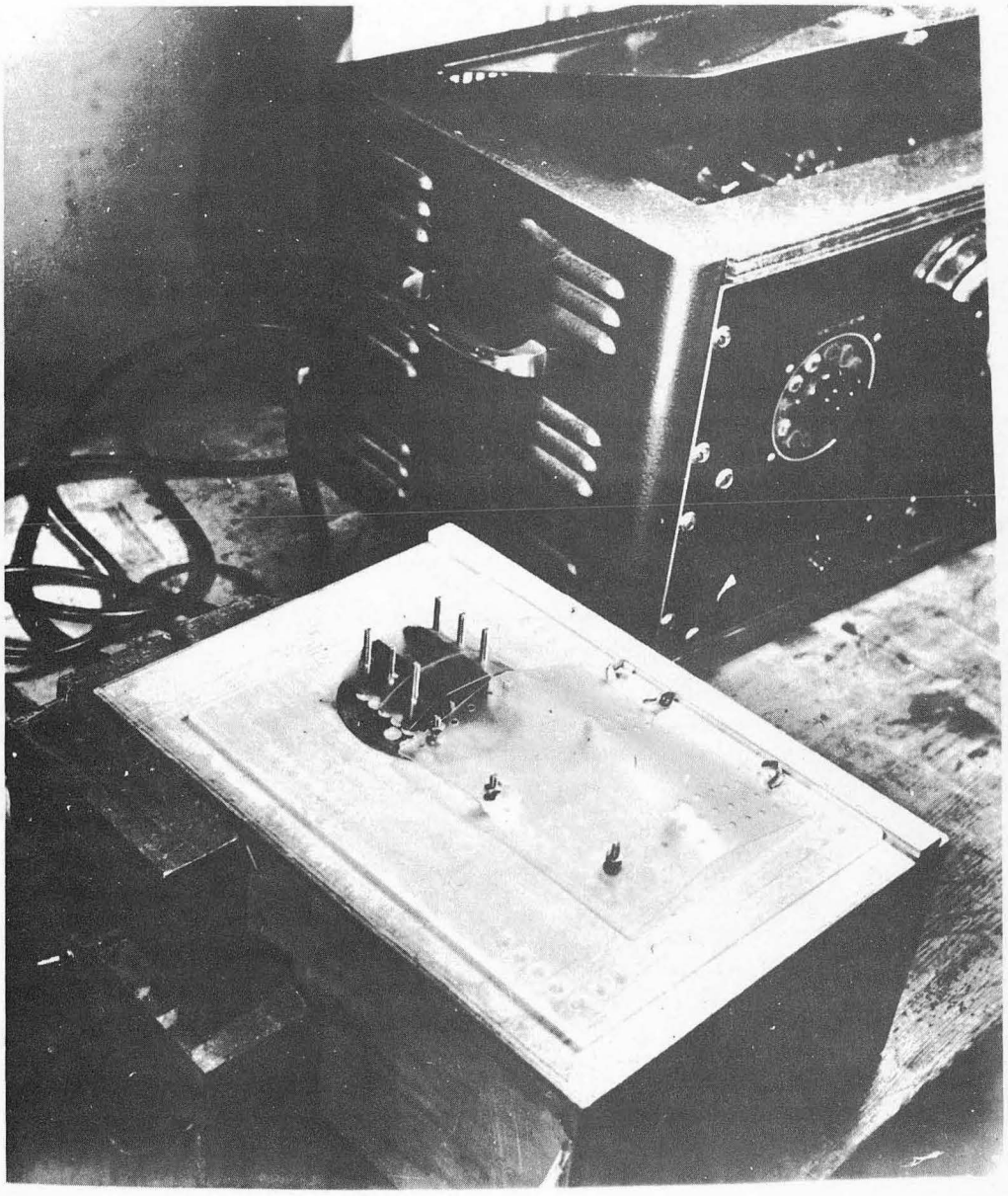

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Fig. 4. In vivo scintillation apparatus showing relationship between animal holder and crystal shield (appears as dark circle in upper third of covering box).

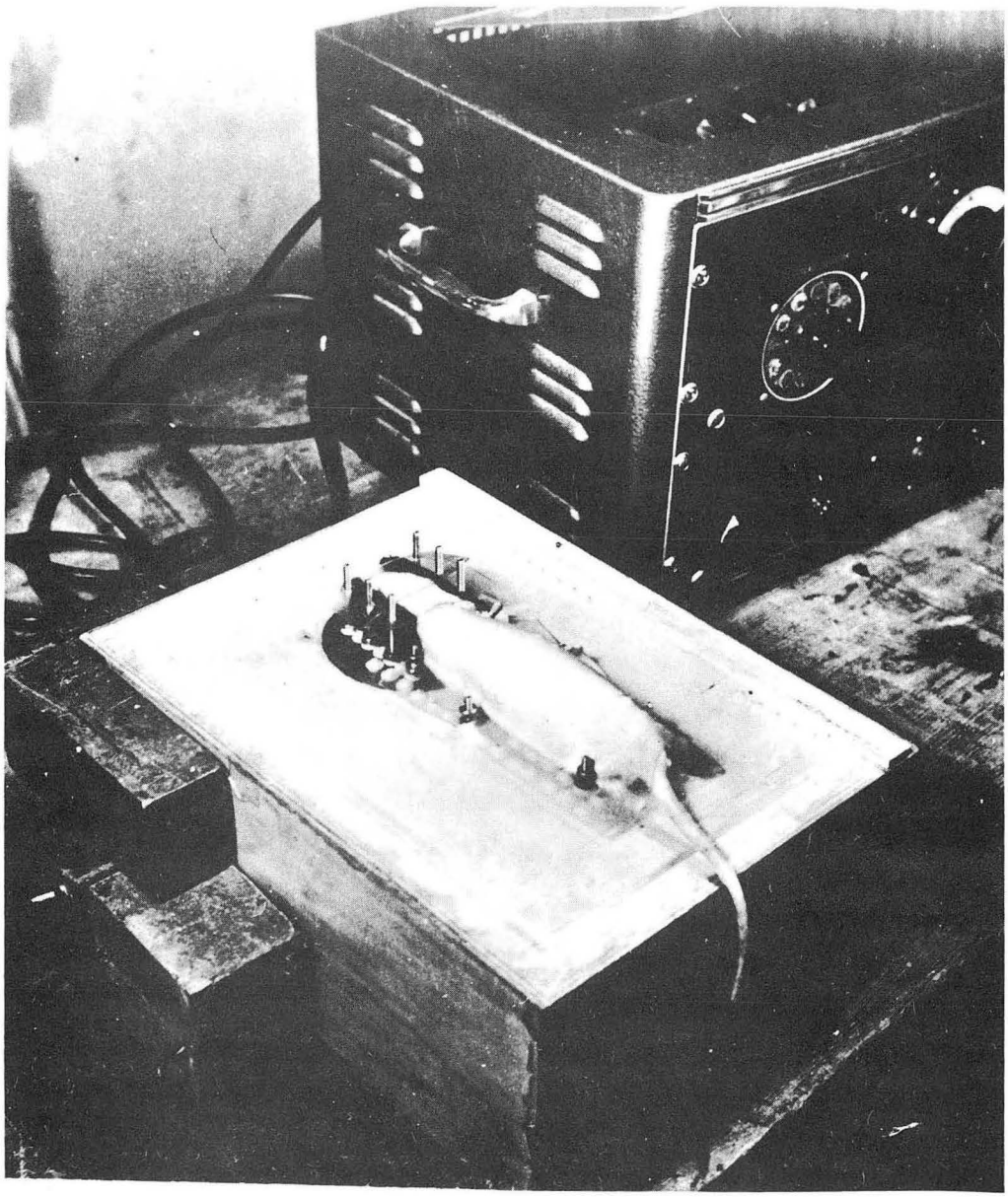

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Fig. 5. In vivo scintillation apparatus showing placement of animal in holder.

The rat is mounted so that the thyroid area is defined by a slot, 0.5 in. by 1.25 in., in the holder. This slot in turn is positioned over the 0.5-in.-diameter aperture in the lead shield above the crystal. The holder is first positioned so that one end of the slot is directly over the crystal and a count is made. The holder is moved in 0.25-in. increments, and counts are taken at each position until the other end of the slot is over the aperture. A sharp peak in counting rate will usually be found at some one position, indicating localization of the thyroid area.

The standard for each run was prepared as follows: Squares 1.25 by 1.25 in. were cut from 0.25-in. Plexiglass. A flat-bottomed hole 0.375 in. in diameter by 0.1875 in. deep was drilled in the center of each square. An aliquot of the standard was placed in the hole with a small amount of KI carrier plus $AgNO_3$. The standard was counted on the rat holder in order to simulate the conditions under which the living animals were counted.

Results with I^{131}

The 16-hour thyroid I^{131} uptake was measured in vivo and, for comparison, by several in vitro methods using the same group of five rats. The methods and recoveries obtained were as follows:

	Counting Method	Average percent of dose
1.	Thyroid area of live animal - In vivo counter.	7.33
2.	Animal sacrificed - Thyroids dissected and mounted on plastic standard holder. Counted with in vivo counter.	7.16
3.	Thyroids mounted as in (2). Counted with scintillation counter described by Jenkins.[2]	7.31
4.	Thyroid homogenized. Aliquot of hemogenate counted with G-M counter.	6.99

The error between the lowest and highest thyroid I^{131} uptakes was 4.8 percent. The above results were considered entirely satisfactory.

The intravenous injection of I^{131} in the external jugular vein was found to be an unsatisfactory preparation for this in vivo counting method. Some of the material may diffuse rapidly into the tissues adjacent to the injection site, leading to an erroneous initial rate of thyroid accumulation. The best results were obtained by intramuscular injection in the hind leg.

[2] K. D. Jenkins, Scintillating Crystal Gamma Counter, University of California Radiation Laboratory Report No. UCRL-1766, May 1952.

Results with At^{211}

The thyroid uptake of intramuscularly injected At^{211} was determined in vivo and compared as above in another group of five rats. In vivo counting was not successful. The tissue uptake of At^{211} is sufficiently large to mask the thyroid uptake for the first 12 hours after injection.[3] The high tissue background made it impossible to localize the thyroid. Operative procedures will be attempted.

Conclusions

1. An in vivo counting apparatus has been developed for the accurate measurement of thyroid I^{131} uptake in rats which are in an entirely physiological state.

2. Measurement of thyroid At^{211} uptake by this method has not been successful.

The advice and assistance of the following are gratefully acknowledged: Kenneth Jenkins, 60-inch Cyclotron staff, Crocker Laboratory, for the design of the photomultiplier circuit; Patricia W. Durbin, Crocker Laboratory; and Dr. R. W. E. Watts, St. Bartholomew's Hospital Medical College, London, England.

[3] J. G. Hamilton, C. W. Asling, W. M. Garrison, and K. G. Scott, "The Accumulation, Metabolism, and Biological Effects of Astatine in Rats and Monkeys," Univ. Calif. Pub. Pharmacol. 2, 283-344 (1953).

THYROID DESTRUCTION BY RADIOACTIVE ELEMENTS ASTATINE-211 AND IODINE-131

R. W. E. Watts*

The actions of lethal and sublethal doses of At^{211} on rats and monkeys as judged by clinical, histological, and haematological criteria have been reported previously (Hamilton, Asling, Garrison, and Scott[1]) (Hamilton, Durbin, and Parrott[1,2,3]). A myxedemalike syndrome was noted in monkeys many months after the administration of At^{211}, but no attempt was made to study precisely the time course of the development of detectable thyroid hormone deficiency or to assess quantitatively its severity.

*Traveling Fellow of the British Postgraduate Medical Federation, University of London. Has been working with C. Willett Asling, Associate Professor of the University of California at Berkeley.

[1] J. G. Hamilton, C. W. Asling, W. M. Garrison, and K. G. Scott, Univ. Calif. Publ. Pharmacol. 2, 283-324 (1953).

[2] J. G. Hamilton, P. W. Durbin, and M. W. Parrott, The 2nd Radioisotope Congress, Oxford, Proceedings Med. and Physiol. Applications 1, 219-231 (1954).

[3] J. G. Hamilton, P. W. Durbin, and M. W. Parrott, J. Clin. Endoc. Met. 14, 1161-1178 (1954).

The objects of the work described here were to study quantitatively the changes which occur in the over-all energy metabolism of rats following a thyroidectomizing dose of At^{211} and to compare these changes with those which occur after a thyroidectomizing dose of I^{131}.

Materials and Methods

Experimental Animals

Adult female Sprague-Dawley rats weighing 200 to 250 grams were used for this work. They were given water and the pelleted diet routinely in use at the Crocker Laboratory ad lib., and allowed to become acclimatized to the prevailing conditions of diet and temperature for two to three weeks before the metabolic rate determinations were started. The circumambient temperature of the room in the Institute of Experimental Biology where these animals were housed during the period of study was 75° F and was thermostatically controlled.

Isotopes and Injections

The At^{211} was prepared by a modification of the procedure described by Garrison, Gile, Maxwell, and Hamilton[4] and was diluted in isotonic NaCl containing 5 mg $Na_2SO_3 \cdot 7H_2O$/ml. The carrier-free I^{131} in the form of NaI was obtained from Oak Ridge National Laboratory and diluted in isotonic NaCl for injection. All the injections were made directly into the external jugular vein under light ether anaesthesia. Twelve rats (average weight 248 g) were given 0.8 μc At^{211}/g body weight, and ten rats (average weight 220 g) were given 10 μc I^{131}/g body weight. Two groups of ten control animals received isotonic saline (0.5 ml/rat).

Metabolic Rate Determinations

The method employed depends upon the measurement of the time required for the animal to consume a measured volume of oxygen from a closed system. The final result was expressed as Cal/m^2 body surface area/hour, the surface area being calculated from the formula

$$\frac{9 \times \sqrt[3]{\text{Body Weight}^2}}{10,000} = \text{Body surface area, } m^2$$

(Contopoulos, Evans, Ellis, and Simpson[5]). The compartment of the apparatus in which the rat is placed was made as small as possible in order to limit the animals' spontaneous movements. Basal conditions were approximated by training the animals to the apparatus, and by depriving them of food, but not of water, for approximately 20 hours before each measurement. The results obtained on normal animals by this method are in satisfactory agreement with those published by workers using alternative methods (Carr and Krantz[6]).

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[4] W. M. Garrison, J. D. Gile, R. D. Maxwell, and J. G. Hamilton, Analyt. Chem. 23, 204-205 (1951).

[5] A. N. Contopoulos, E. S. Evans, S. Ellis, and M. E. Simpson, Proc. Soc. Exp. Biol. 86, 729-733 (1954).

[6] C. J. Carr and J. C. Krantz, "Metabolism in the Rat in Laboratory Investigation," Philadelphia: Lippincott (1942).

Repeated metabolic rate determinations were made on both the experimental and control groups of animals before the injections were made (see Figs. 6 and 7) in order to determine the range of spontaneous variation in the average metabolic rate of each group that might be expected under the prevailing laboratory conditions.

Results

The average metabolic rates, together with the range ± the standard deviation, for each group of experimental and control animals are presented in Figs. 6 and 7. In Fig. 8, the ratio (average metabolic rate for experimental group) per (average metabolic rate for the corresponding control group) has been calculated for each time interval studied, and the results obtained with the At^{211} and I^{131} treated groups of rats are compared directly.

Five of the twelve animals given At^{211} died between eight and seventeen days after the administration of the isotope, and although the metabolic rates of these animals did not differ appreciably from the other members of the group, the mean values given are based only on observations made on the animals which subsequently survived the whole course of the experiment. The mean metabolic rate of the At^{211}-treated animals decreased sharply between six and eight days after the administration of the isotope, subsequently decreasing gradually to between 60 and 70 percent of the corresponding control value.

There were no deaths among the I^{131}-injected animals. The average metabolic rate of this group began to decrease between eight and twelve days after the administration of I^{131}, subsequently decreasing more gradually and fluctuating more widely than the average metabolic rate of the At^{211}-injected group.

Discussion

The doses of At^{211} and I^{131} employed in these experiments were judged on the basis of previous studies to be sufficient to produce complete radiothyroidectomy. Ideally, it would have been desirable to have used doses of At^{211} and I^{131} which were also comparable from the point of view of their systemic toxicity. This was not possible, owing to the greater toxicity of At^{211} than I^{131}, the median lethal dose at 60 days (MLD_{60}) being 1.12 μc At^{211}/g body weight and 85 μc I^{131}/g body weight for the rat (Hamilton et al. 1954[2]). The autopsy changes observed in the At^{211}-treated rats that died were similar to those which Hamilton et al. (1954)[2] attributed to the effects of internal irradiation by At^{211}. The fact that it was not possible to predict from a study of the animals' metabolic rates which members of the At^{211}-injected group would succumb to radiation effects supports the view that the differences observed between the At^{211}-injected and the I^{131}-injected animals were not attributable to the more severe generalized internal irradiation to which the former group were exposed.

The radioactive decay of At^{211} (half life 7.3 hours) involves the emission of 5.86-Mev alpha particles and 80-kev x-rays, whereas I^{131} decays by the emission of beta particles (average energy 0.2 Mev) and a cascade of gamma rays. Thus, for equal numbers of At^{211} and I^{131} atoms in vivo, At^{211} results in a greater but more localized linear-energy transfer over a shorter period

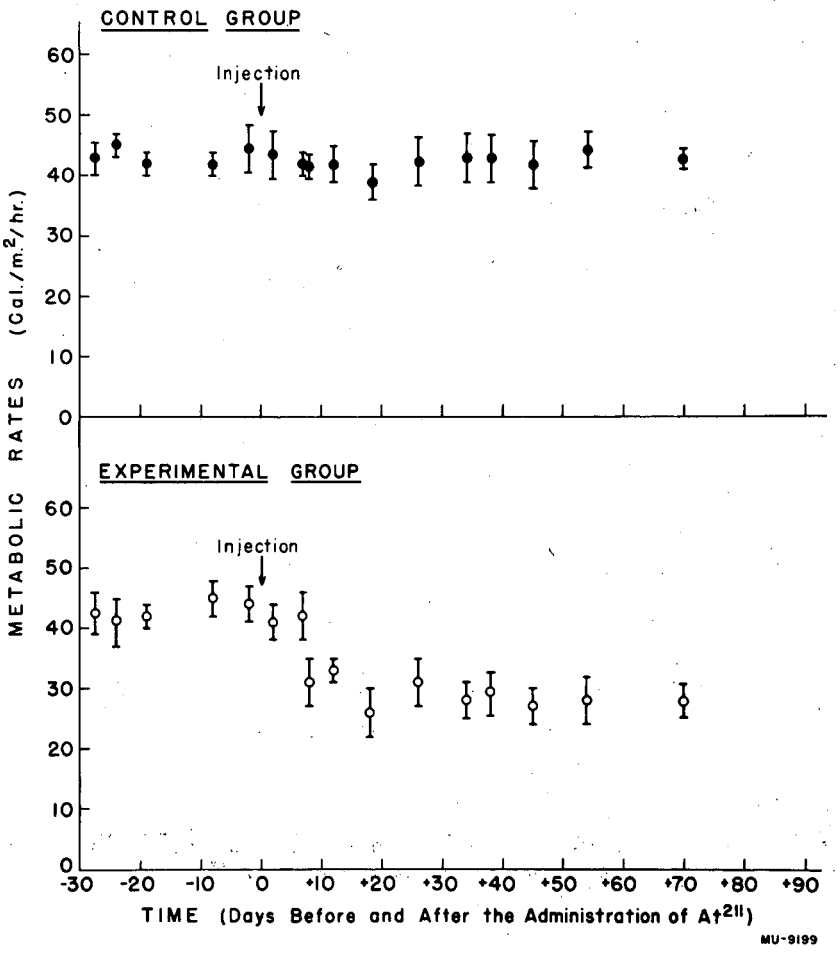

Fig. 6. Metabolic rates of At^{211}-treated rats (average values ± 1 x standard deviation).

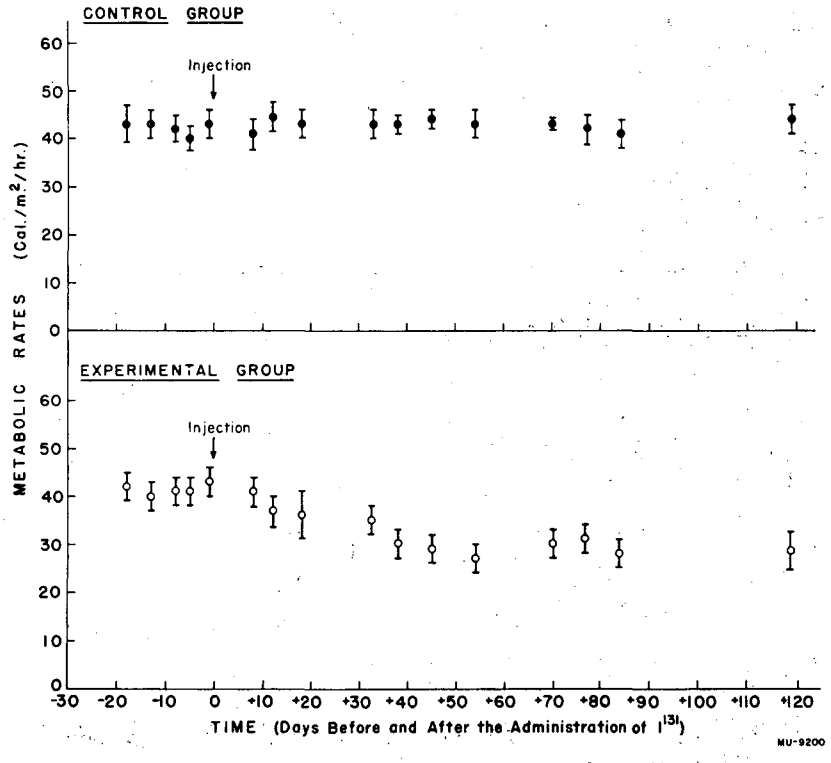

Fig. 7. Metabolic rates of I^{131}-treated rats (average values ± 1 x standard deviation).

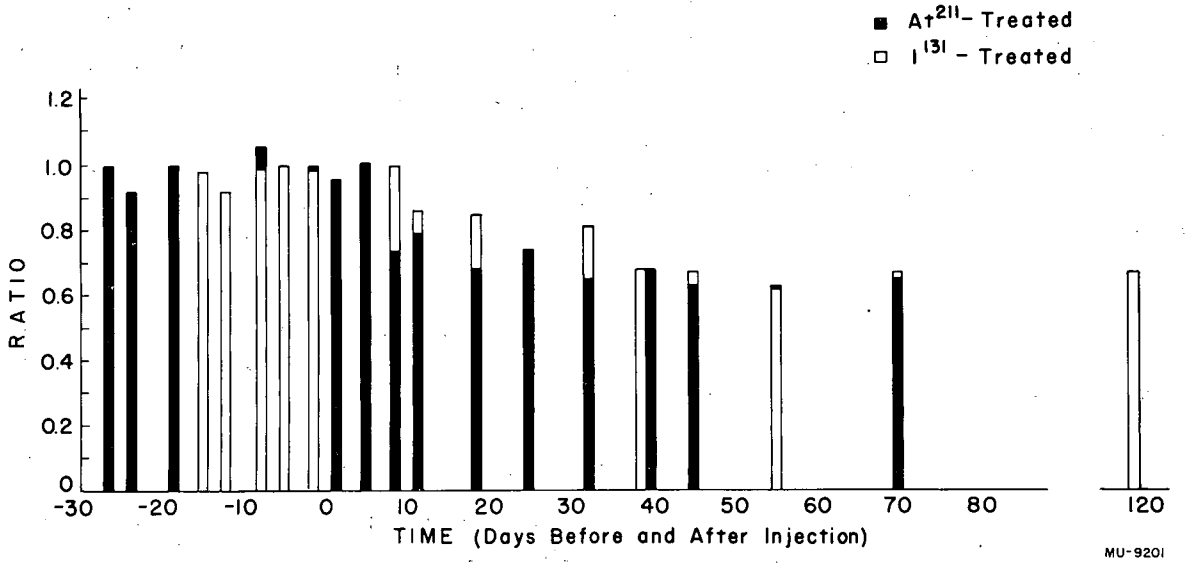

Fig. 8. Comparison of the ratio (average metabolic rate of treated animals/average metabolic rate of untreated animals) for At^{211} treated and I^{131} treated rats.

of time than does I^{131}. It is not possible to estimate the ratio of the numbers of At^{211} and I^{131} atoms present in the thyroid glands of the two groups of experimental animals in the present study. The dose of At^{211} used in terms of microcuries (i.e., number of nuclear disintegrations occurring in unit time) was considerably smaller, however, than the dose of I^{131} used, and it is known (Hamilton et al., 1953[1]) that a smaller proportion of a dose of At^{211} than of the same dose of I^{131} is taken up by the thyroid gland. It is unlikely, therefore, that the thyroids of the At^{211}-treated animals actually contained more radioactive atoms than the thyroids of the I^{131}-treated group at any given time. In spite of this, thyroid function, as judged by the animals' metabolic rates, decreased more abruptly in the former than in the latter group--a finding which could be attributed to more acute glandular destruction, i.e., to a greater dissipation of energy within the gland, over a relatively short period of time, and hence correlated with the different nuclear properties of At^{211} and I^{131}.

Summary

1. The time course of the development of hypothyroidism in rats, as judged by the animals' metabolic rates, following the administration of thyroid destructive doses of At^{211} and I^{131}, has been studied.

2. The metabolic rate decreased abruptly to about 75 percent of the control value between six and eight days after the administration of 0.8 μc At^{211}/g body weight. A further decrease to about 65 percent of the control level occurred over a period of approximately one month.

3. Slowing of the metabolic rate was first observed between eight and twelve days after the administration of I^{131} (10 μc/g body weight). The metabolic rate decreased more slowly in the I^{131}-treated than in the At^{211}-treated animals.

4. It is suggested that the differences that have been observed after the administration of At^{211} and I^{131} may be correlated with the different nuclear properties of the two isotopes.

Acknowledgments

The writer is pleased to express his indebtedness to Professor Joseph G. Hamilton and Dr. Patricia W. Durbin for helpful discussions during the course of the present work, and to Professor Joesph G. Hamilton and Professor C. Willett Asling for professional hospitality during part of the tenure of a Traveling Fellowship awarded by the British Postgraduate Medical Federation, University of London. The At^{211} was prepared by Mr. G. Bernard Rossi and the staff of the 60-inch Cyclotron; the radiochemical separation of this isotope from the cyclotron target was performed by Mr. Marshall W. Parrott. Sincere thanks are tendered to all these gentlemen.

STUDIES ON THE CHEMICAL STATE OF ASTATINE-211 IN THE THYROID GLANDS OF NORMAL AND PROPYL-THIOURACIL-TREATED RATS

Patricia W. Durbin, Nylan Jeung, Marilyn Williams, and Ruth Newman

An investigation of the chemical state of At^{211} in the thyroid gland, and the possibility that it is organically bound, was stimulated by two observations: (a) the concentration of At^{211} in the thyroid gland of the rat 24 hours after its intravenous administration is several hundred times that in the plasma,[1,2] and (b) the activity-time relationship of the thyroidal accumulation of At^{211} closely resembles that obtained for I^{131} under similar conditions.[1] Some preliminary experiments suggested that the At^{211} was closely associated with the thyroid protein.[3] The experiments reported here were designed to shed some light on the fate of At^{211} in the normal thyroid gland, on the intriguing enhancement of the thyroidal accumulation of At^{211} in propyl thiouracil-treated animals,[2,4] and on the further enhancement due to withdrawal of PTU described in the preceding Medical Physics Quarterly Report, UCRL-2881, October, November, December 1954.

Female Sprague-Dawley rats 60 to 65 days old were used throughout unless otherwise specified. They were maintained for at least two weeks prior to use on Purina Laboratory Chow and tap water. Groups of 10 to 20 rats were pretreated as follows: (1) controls, no pretreatment; (2) KSCN, the animals received a single subcutaneous injection of 0.2 ml of a 10 percent solution of KSCN 1.5 hours before sacrifice; (3) PTU, the drinking water was replaced by a 0.1% solution of 6n-propyl thiouracil for 11 days prior to radiohalogen administration; (4) off-PTU, the PTU solution was given for eight days, followed by tap water for three days; (5) PTU + KSCN, the animals were given the solution for 11 days and received a single subcutaneous injection of 0.2 ml of a 10% solution of KSCN 1.5 hours before sacrifice on the eleventh day. All the rats received 10 μc of At^{211} intravenously. The control, PTU, and off-PTU groups also received 2 μc of I^{131} intraperitoneally. The PTU and PTU + KSCN groups were given the PTU solution until sacrificed.

Eighteen hours after the administration of the radiohalogens the animals were sacrificed with chloroform, the thyroids were removed, weighed, and homogenized in 1 ml of cold 1% saline. The homogenates were centrifuged, the cellular debris was washed with 1 ml of 1% saline, centrifuged, and discarded. The saline-soluble homogenate and saline wash were combined. In order to determine the total At^{211} and (or) I^{131} in the thyroid glands, an aliquot was taken from each thyroid homogenate. These samples were plated on and in bottle caps for assay as described elsewhere.[5] The cellular debris was

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[1] J. G. Hamilton, C. W. Asling, W. M. Garrison, and K. G. Scott, Univ. of Calif. Publ. in Pharmacol. 2, No. 21, 283-344 (1953).

[2] C. J. Shellabarger and J. T. Godwin, J. Clin. Endocrinol. and Metabolism 14, 1140-1160 (1954).

[3] P. C. Wallace and K. G. Scott, unpublished data.

[4] P. W. Durbin, J. G. Hamilton, and M. W. Parrott, Proc. Soc. Exptl. Biol. Med. 86, 369 (1954).

[5] P. W. Durbin, J. G. Hamilton, and M. W. Parrott, The Codetermination of I^{127}, I^{131}, and At^{211} in Tissue, University of California Radiation Laboratory Report No. UCRL-2792, Nov. 1954.

not assayed, because pilot studies showed that less than five percent of the total At^{211} or I^{131} in the thyroid gland remained in this fraction after thorough homogenizing and washing. In order to eliminate self-absorption errors, alpha-counting standards of saline homogenate, trichloroacetic acid, and $(NH_4)_2SO_4$ precipitates were prepared by processing nonactive thyroids, both normal and PTU-treated, in exactly the same manner as those under study with the addition of an aliquot of the standard just prior to plating. Some of the thyroid homogenates from each group were added to either an equal volume of 20% TCA or an equal volume of saturated $(NH_4)_2SO_4$. After standing at room temperature for 15 to 30 minutes the TCA and $(NH_4)_2SO_4$ precipitates were collected by centrifugation and washed once with 1 ml of either 10% TCA or half-saturated $(NH_4)_2SO_4$. The supernatants and wash were discarded. The precipitates were broken up and transferred to tinned bottle caps with distilled water and were prepared for assay by the usual methods.[5] The At^{211} alpha activity was measured with a continuous-flow argon-filled ionization chamber, and the I^{131} was assayed on either a G-M or a scintillation counter, depending on the level of activity present.

Results

The 17.5-hour thyroid uptakes of At^{211} and I^{131} under the conditions described in the previous section are shown in Table IV. Although the percent uptake of At^{211} in the PTU-treated rats was twice that of the controls, the concentration of At^{211} in percent per gram of thyroid for this group is not different from the control values, an observation that does not agree with previous findings.[2,4] This may be due in part to the new diet, Purina Chow, which contains only half as much iodine as did "Diet 14," used prior to December 1954 and to the time interval tested. The withdrawal of PTU as shown by the off-PTU values continued to enhance both the total accumulation of At^{211} and its thyroid concentration despite the change in diet and time interval tested. A single subcutaneous injection of KSCN 1.5 hours before sacrifice reduced the thyroidal At^{211} accumulation of PTU-treated rats to very nearly the control level.

The percent of soluble radiohalogen associated with the thyroid protein of both normal and PTU-treated rats is shown in Table V. At the time interval studied, both TCA and half-saturated $(NH_4)_2SO_4$ precipitated nearly all of the I^{131} present in the thyroid glands of the control animals. In the PTU-treated rats neither of these reagents precipitated more than 25% of the I^{131} present. There were no differences observed between the results obtained for the PTU and off-PTU groups. A single injection of KSCN 1.5 hours before a 17.5-hour At^{211} uptake seems to change the behavior of PTU-treated thyroids with $(NH_4)_2SO_4$ so that it more nearly resembles the normal pattern.

Discussion

The results obtained for the behavior of I^{131} in normal and PTU-poisoned thyroids, with the two reagents tested, agree quite well with those obtained by Chaikoff and Taurog.[6] For this reason it is felt that, despite the small numbers of samples involved in some of the experiments, the data presented are fairly

[6] I. L. Chaikoff and A. Taurog, Ann. N. Y. Acad. Sci. 50, 279 (1949).

Table IV

The effects of pretreatment with PTU and KSCN on the 17.5-hour thyroidal accumulation of intravenously administered At^{211} and intraperitoneally administered I^{131} in 60-day-old female Sprague-Dawley Rats. Values are expressed as percent of administered dose and are presented with the standard error of the mean.

Group and Group Number	No. of Rats	At^{211}		I^{131}	
		%/organ	%/gram	%/organ	%/gram
Control (1)*	20	0.78 ± 0.03	50.6 ± 2.4	12.6 ± 0.69	811 ± 52
PTU(3)	21	1.53 ± 0.06	47.0 ± 2.1	0.42 ± 0.03	13.3 ± 1.0
Off-PTU(4)	22	2.98 ± 0.16	83.0 ± 4.0	0.45 ± 0.03	13.7 ± 2.4
KSCN (2)	8	0.56 ± 0.04	34.8 ± 1.6	---	---
PTU+KSCN (5)	10	0.59 ± 0.04	14.7 ± 0.9	---	---

*Refer to group number on page 30 of text for description of pretreatment of rats.

Table V

The extent of association of At^{211} with the protein of the thyroid gland as compared to I^{131} 17.5 hours after administration. With the exception of the PTU + KSCN group, each rat received both At^{211} and I^{131}. Values are presented with the standard error of the mean and are expressed as percent of soluble halide in the thyroid gland precipitatable with either 10% trichloroacetic acid or half-saturated $(NH_4)_2SO_4$.

Group and Group Number	Percent Soluble Halide in Thyroid Gland Precipitated by: 10% TCA			$(NH_4)_2SO_4$		
	No. of Thyroids	At^{211}	I^{131}	No. of Thyroids	At^{211}	I^{131}
Control (1)*	8	90.0 ± 5.7	93.0 ± 5.1	6	69.9 ± 4.3	97.5 ± 2.6
PTU (3)	7	90.0 ± 5.7	21.8 ± 1.8	7	44.3 ± 5.7	24.9 ± 6.7
Off-PTU (4)	4	88.3 ± 5.6	27.2 ± 5.9	4	45.5 ± 6.0	29.3 ± 6.6
PTU + KSCN (5)	4	72.4 ± 3.4	---	4	71.9 ± 1.1	---

*Refer to group numbers on page 30 of text for description of pretreatment of rats.

reliable. There is radioautographic evidence that at 18 to 24 hours after its administration, At^{211}, like I^{131}, is found mainly in the secreted protein, the "colloid," of normal thyroid,[1] and the observation that nearly all the At^{211} is carried in the thyroid protein when it is denatured with TCA would seem to bear this out. In the "salting out" of the protein, specifically the globulin fraction, with half-saturated $(NH_4)_2SO_4$ (which is a dehydrating, rather than a denaturing process), only 70% of the At^{211} followed the protein. The self-absorption of the alpha particles in the salt that accompanies the protein precipitated with $(NH_4)_2SO_4$ was partially taken into account by the preparation of special standards described in the section on methods; however, uneven distribution of the salt after drying could introduce large errors in assay. Further experiments, in which only At^{211} is given and the activity of all samples is measured by scintillation counting of the At^{211} x-rays, are planned to check whether this difference in the behavior of the At^{211} in normal thyroid with the two reagents tested is real or technical.

The difference between the percent of thyroid At^{211} precipitated by TCA and by "salting out" in the PTU-treated rats is sufficiently great, and the quantity discharged from these thyroids by KSCN is also sufficiently great, to permit the conclusion that the accumulated At^{211} is present as inorganic At^{211}, or that its association with the thyroid protein is an extremely labile one. Further substantiation of this can be found in Table VI (in a following section), in which it is shown that At^{211} accumulated by the thyroids of PTU-treated rats was more readily leached by all the fixatives tested. There is evidence that the prolonged administration of PTU results in a radical change in the nature of the thyroid protein,[7] which may have some bearing on the problem at hand, if At^{211} is not organically bound in the ordinary sense but--because of its semimetallic properties in some valence states--is chemically adsorbed onto the thyroid protein.

DIFFUSION STUDIES WITH ASTATINE-211

Patricia W. Durbin

Dialysis has proved to be one of the most useful tools in the study of the organic binding of iodine by the thyroid gland. In order to evaluate accurately experiments involving the dialysis of thyroid preparations containing At^{211} under a given set of experimental conditions, it was necessary to determine the rate of diffusion of inorganic At^{211}. Because absolute diffusion rates are quite sensitive to changes in temperature and the concentration of various constituents, some of the variables were eliminated by measuring the ratio of the diffusion rate of At^{211} to that of I^{131}.

Methods

The apparatus consisted of Visking sausage-casing bags supported by short pieces of cylindrical Pyrex tubing, 2 cm i.d., one end of which was slightly flared. The casing was immersed in water until softened and workable, and

[7] W. T. Salter, Ann. N. Y. Acad. Sci. 50, 358 (1949).

one end was knotted tightly to form a bag. The open end of the casing was passed through the glass cylinder towards the flared end, was rolled over the outside so that about one-half inch overlapped the glass, and was secured with a rubber band.

Five microcuries each of At^{211} and I^{131} were mixed thoroughly in 100 ml of a 1% saline solution containing 0.0012 M KI and 0.004 M Na_2SO_3. Equal volumes of the At^{211} and I^{131} solution were pipetted into four bags, which were suspended in a crystallizing dish containing two liters of the same 1% saline solution. This solution was used throughout, so that the only difference between the inside and outside media was the presence of the radiohalogens. Because the addition of iodide ion would tend to minimize differences between the diffusion rates of I^{131} and At^{211} due to differences in the initial concentration gradients, in future experiments thyroids would be homogenized in one percent saline, and the addition of a reducing agent, Na_2SO_3, would maintain the At^{211} as At^-. To speed up the diffusion rates the outside solution was stirred constantly and was replaced by fresh solution at the end of the first hour. The bags themselves were agitated after each sample was taken. A 0.2-ml sample was removed from each bag every ten minutes for the first hour and every thirty minutes until the end of the run, usually three to four hours. The samples were plated on tinned bottle caps as reported elsewhere,[1] and were assayed for both At^{211} and I^{131} radioactivity. The At^{211} assays for all of the samples from any given run were all completed within thirty minutes; corrections for decay were not made. The I^{131} was always assayed on the following day to allow for almost complete decay of the At^{211}.

Results and Discussion

A semilog plot of At^{211} or I^{131} in the inside medium in counts per second per unit volume versus time in minutes invariably resulted in curves which could be resolved into one rapid and one slow component. The slow component usually appeared at the end of about three hours, and may be attributed either to errors of measurement (the counting rates were less than 10 cps), or to the sizable decrease in concentration gradient. Because of the uncertainty, only the rapid components are discussed here. The ratio of the diffusion half-times $T_{1/2}(I^{131})/T_{1/2}(At^{211})$ has been determined for a total of seven runs, and equals 0.71 ± 0.01. The diffusion coefficient, D, is obtained from $D = \ln 2/T_{1/2}$, and the ratio $D_I:D_{At}$ is then 1.41. For gases D is inversely proportional to the square root of the molecular weight.[2] The use of equations derived from kinetic theory have been widely applied to the problem of aqueous solutions. In order to test the validity of the measurements the theoretical ratio of the diffusion coefficients for At^{211} and I^{131} was calculated from

$$D = \frac{k}{\sqrt{M}} \text{ or } \frac{DI}{D_{At}} = \frac{\sqrt{M_{At}}}{\sqrt{M_I}}; \quad D_I/D_{At} = 1.29$$

The measured value 1.41, therefore, agrees as well as might be expected with the theoretical one, 1.29.

---

[1] P. W. Durbin, J. G. Hamilton, and M. W. Parrott, The Codetermination of I^{127}, I^{131}, and At^{211} in Tissue, University of California Radiation Laboratory Report No. UCRL-2792, Nov. 1954.

[2] S. Glasstone, Textbook of Physical Chemistry, 2nd ed., Van Nostrand Co., Inc., New York, 1946, 254.

STANDARDIZATION OF TECHNIQUES IN THE PREPARATION OF RADIOAUTOGRAPHS WITH ASTATINE-211 AND IODINE-131

Muriel Johnston, C. Willett Asling, Patricia W. Durbin, and Joseph G. Hamilton

With the beginning of the series of experiments using PTU-treated rats, the problem of leaching of At^{211} from fixed thyroid tissue again arose. (See Quarterly Report for April, May, June 1952, UCRL-1922). To all appearances, PTU-treated animals fail to do as successful a job of binding At^{211} into the thyroid tissue as do normal animals. As a result, an experiment was run to determine the most satisfactory fixative for both normal and PTU-treated thyroids. The following four fixatives were tried on both normal and PTU-treated thyroids from animals that had been given At^{211}: (a) 10% neutral formalin, (b) Bouin's, (c) alcoholic formalin, and (d) acetic formalin, Tellyeniczky's fluid. After nine hours of fixation, the thyroids and the fluid fixatives were counted separately on a scintillation counter. From the counting results, as can be seen from Table VI, Bouin's was far superior for holding down the amount of leaching. Consequently, Bouin's will be used for all future thyroid histology.

A previous experiment had been run to determine the efficiency of an alcohol-xylol dehydration series versus a Dioxane series for the preparation of thyroids containing either At^{211} or I^{131} by the paraffin method. Dioxane was shown to leach little or none of these radiohalogens, and as a result, paraffin embedding is always preceded by the use of this dehydration and clearing medium.

Further information was sought on which preparatory technique might be the most expeditious in the readying of sections for radioautographs. The time-saving features of the frozen technique bring it to mind first, particularly in dealing with the short half-life (7.3 hours) of At^{211}.[1] The size of rat thyroids, however, is sufficiently small to allow the use of short time intervals for the dehydration and clearing that are necessary in the paraffin technique. Thus, the paraffin technique can be utilized, and it delays the beginning of autograph exposure by only six to seven hours beyond the time that frozen sections can be readied for exposure. This group has decided to use the paraffin technique almost exclusively for the following reasons: (a) section thickness can be more accurately controlled (6μ versus 10μ or more for frozen); (b) nuclear detail is usually greater; (c) more colloid remains in the section, probably owing to the added support of the paraffin; and (d) there is apparently less migration of At^{211} into the area surrounding the section on the film, especially in the PTU preparations.

Examples are shown in Figs. 9 through 14.

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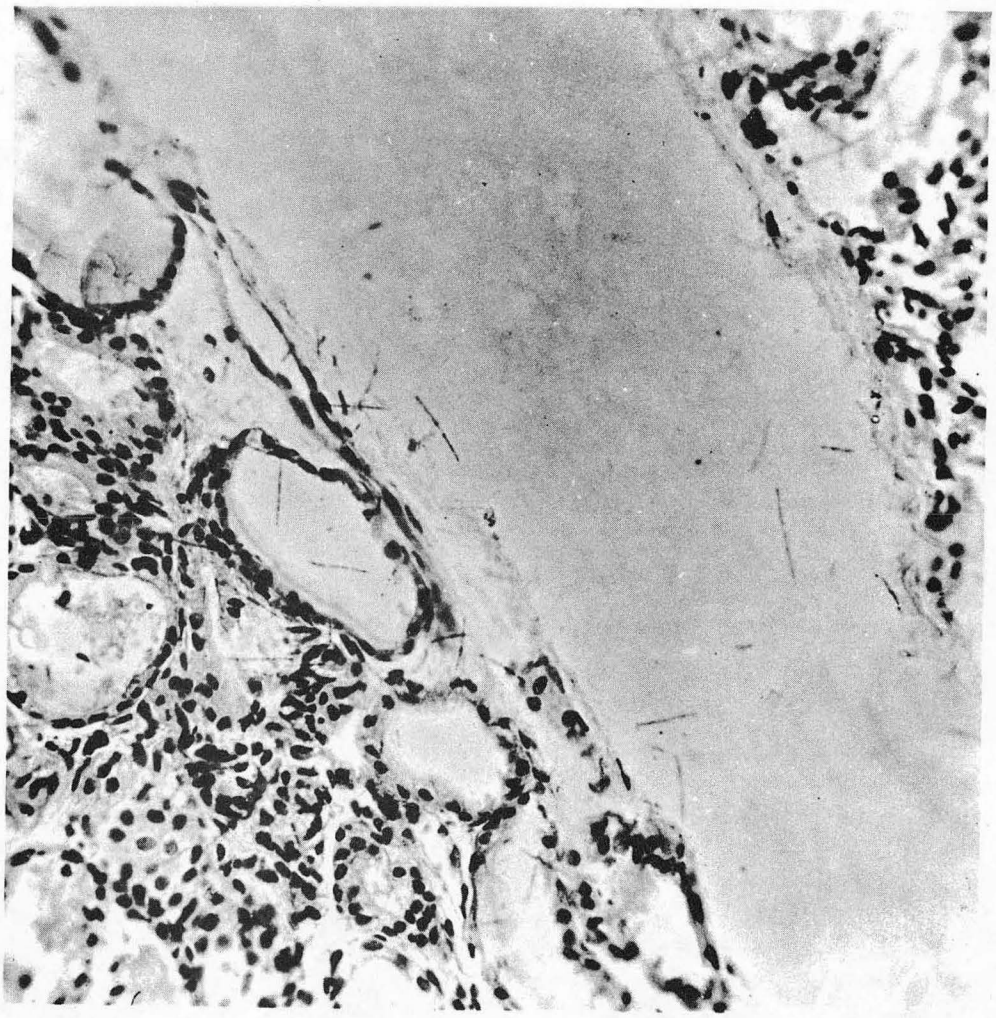
[1] C. J. Shellabarger and J. T. Godwin, J. Clin. Endocrinology and Metabolism 14, 1149-1160 (1954).

Table VI

The leaching of At^{211} from the thyroid glands of normal and PTU-treated rats in formaldehyde and alcoholic-formaldehyde fixatives. Thyroids were removed seven hours after At^{211} administration and were fixed for eight hours. Values are expressed as percent of total thyroid At^{211}.

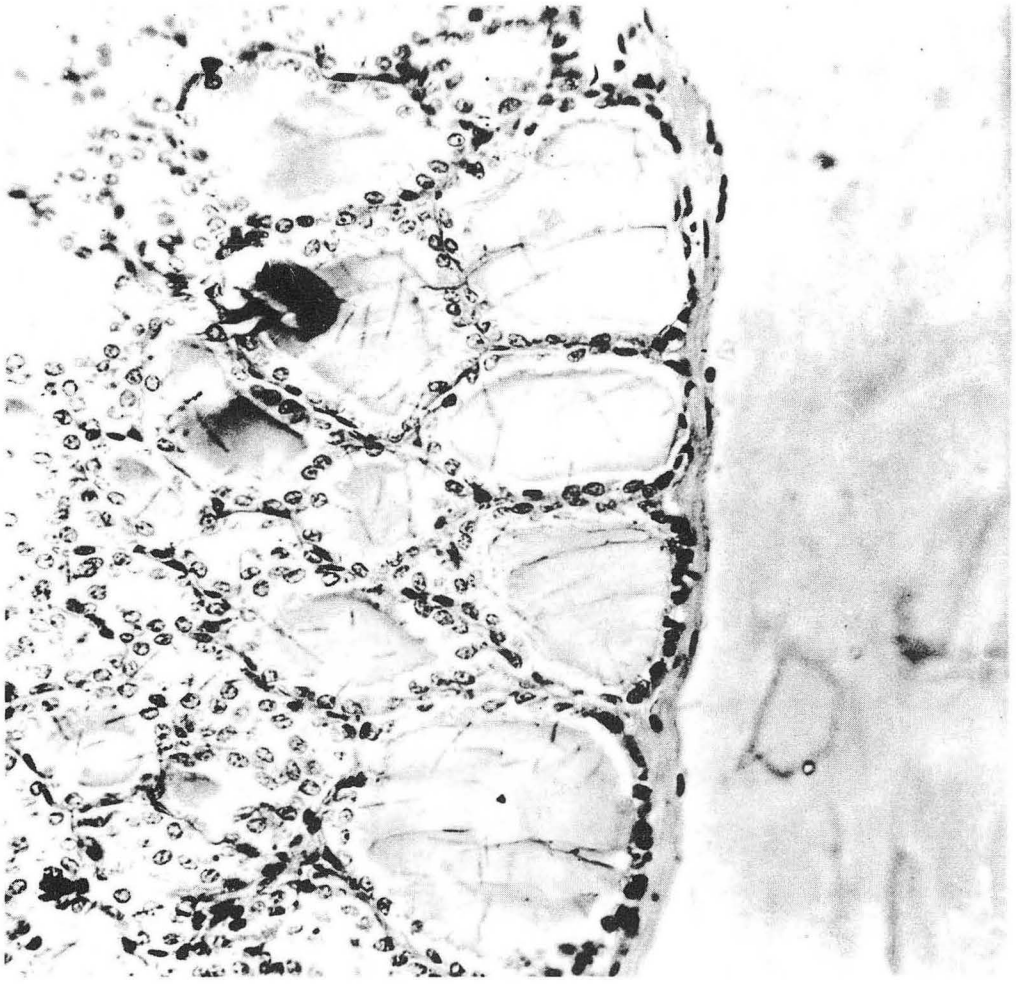
Fixative	Percent Leached	
	Normal Thyroid	PTU Thyroid
10% Formalin*	7.9	11.5
10% Formalin in 95% ethanol	57.5	85.9
5% Formalin and 5% glacial acetic acid in 70% ethanol	17.1	16.5
Bouin's fluid (25% formalin and 5% glacial acetic acid in saturated picric acid)	3.3	2.9

*Percent by volume in all cases.



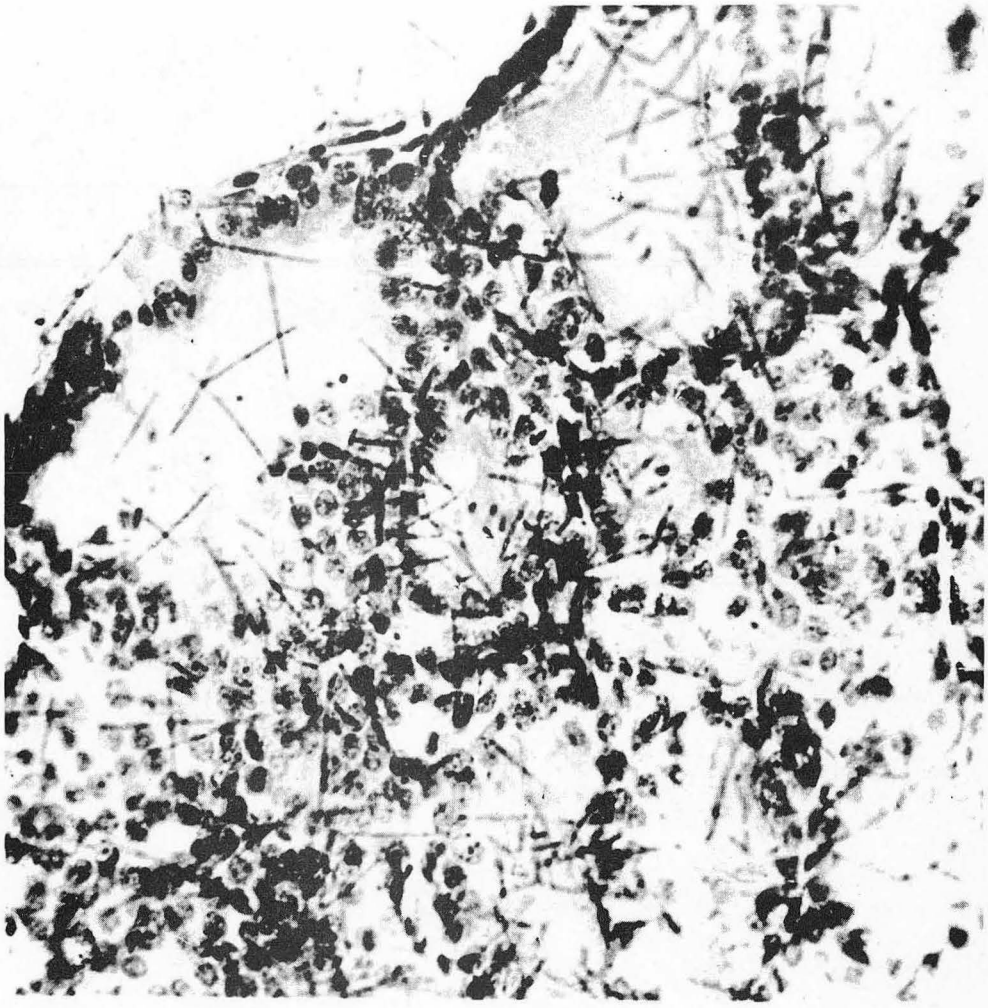
ZN-1276

Fig. 9. Paraffin section of normal rat thyroid 6μ in thickness, fixed in 10% neutral formalin. x 310. Compare with Fig. 10.



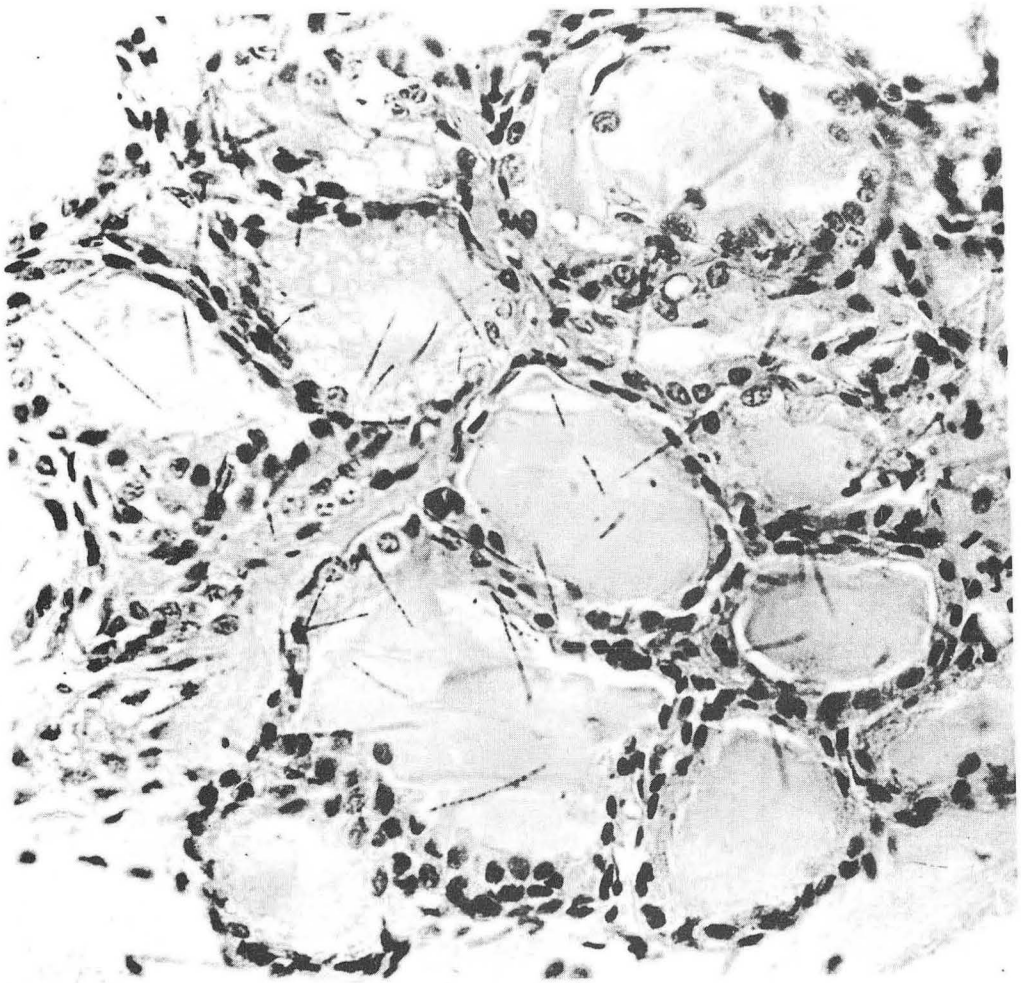
ZN-1277

Fig. 10. Paraffin section of normal rat thyroid 6μ in thickness, fixed in Bouin's. x 310. Note greater nuclear detail than in Fig. 9, and the presence of more colloid in the follicles.



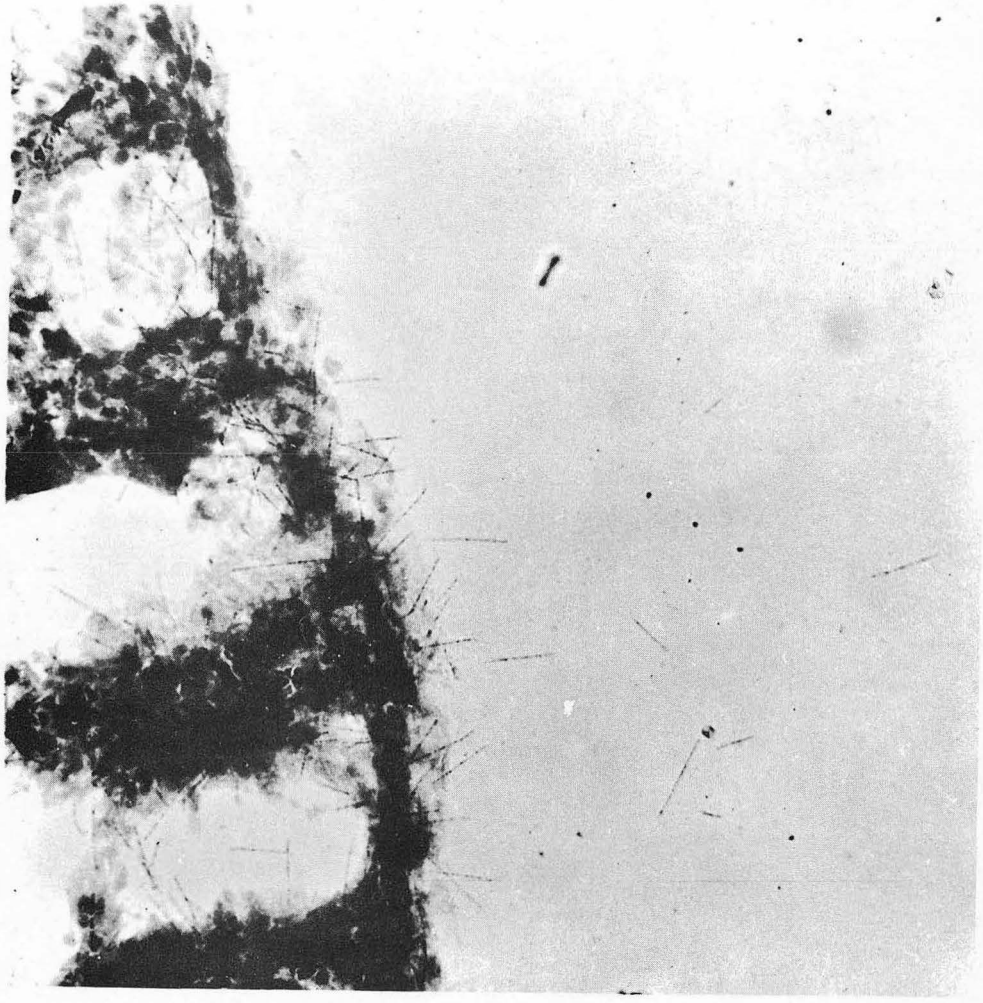
ZN-1278

Fig. 11. Frozen section of normal rat thyroid approximately 10μ in thickness, fixed in Bouin's. x 400. Compare with Fig. 12.



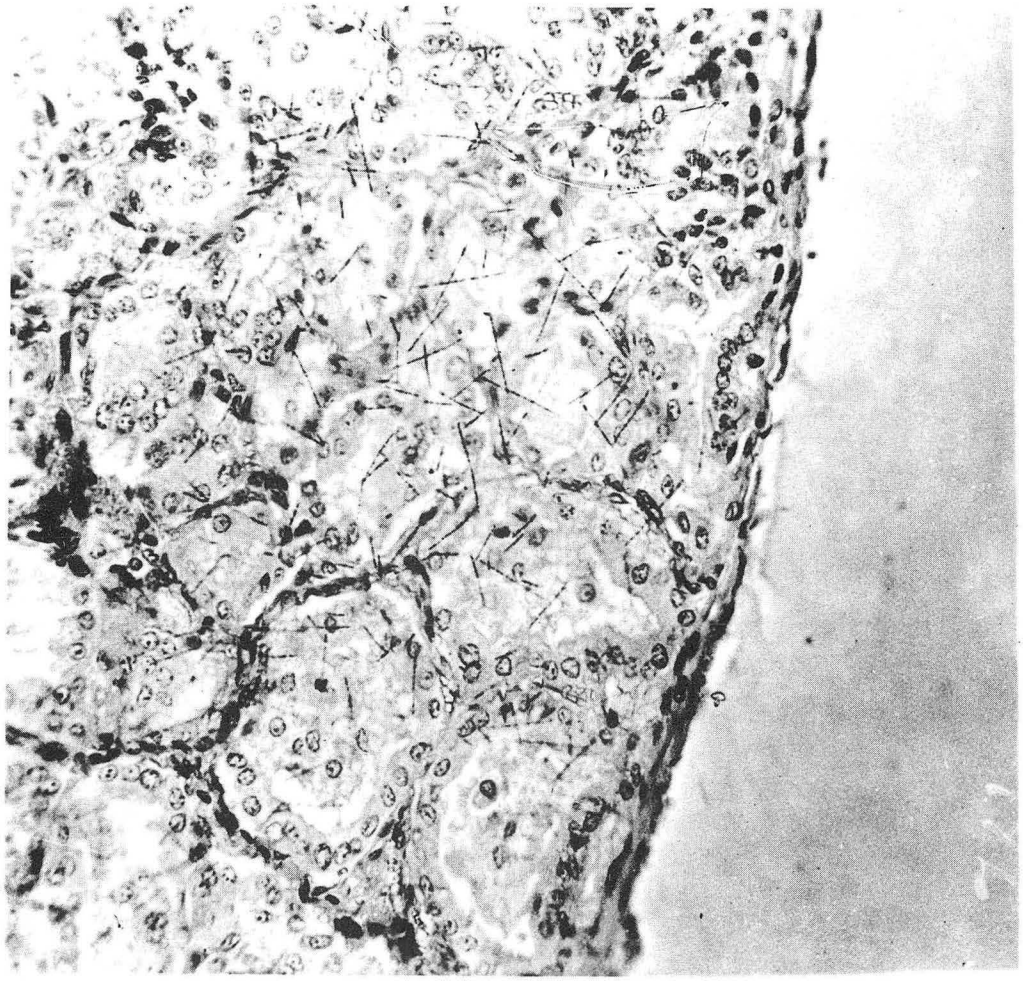
ZN-1279

Fig. 12. Paraffin section of normal rat thyroid 6μ in thickness, fixed in Bouin's. x 400. Compared with Fig. 11, nuclear detail is more evident, as is the comparative thinness of the section, and colloid is better defined in the follicles. Fewer tracks are evident in this plate than in Fig. 11, presumably due to the time lag in preparing paraffin sections.



ZN-1280

Fig. 13. Frozen section of PTU-treated rat thyroid approximately 10μ in thickness, fixed in 10% neutral formalin. x 310. Note tracks wandering from edge of tissue (leached At^{211}), lack of detail, and thickness of section. Compare with Fig. 14.



ZN-1281

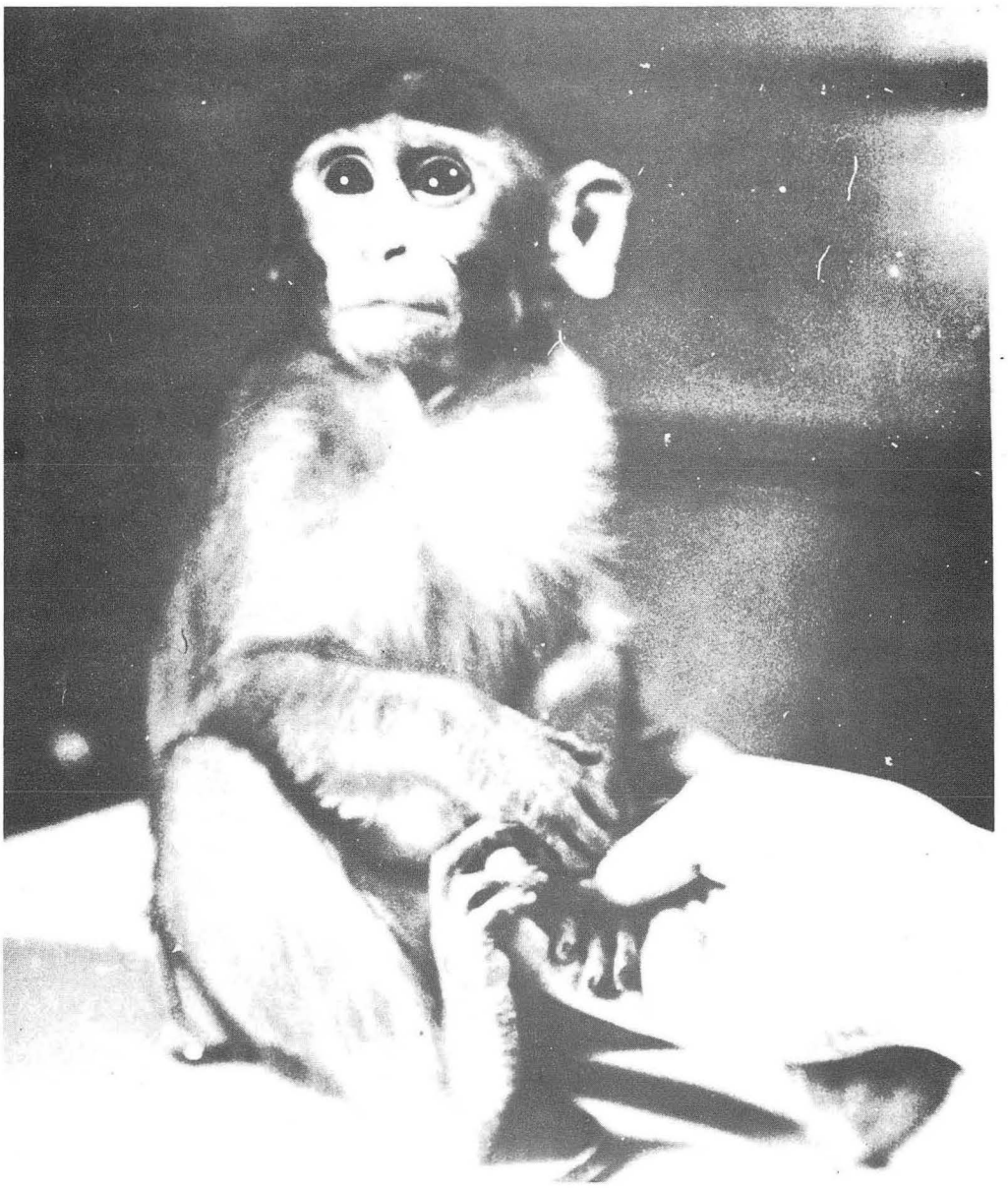
Fig. 14. Paraffin section of PTU-treated rat thyroid 6μ in thickness, fixed in Bouin's. x 310. Note nuclear detail, edge of tissue clear of tracks, and uniform thinness of section. Compare with Fig. 13.

TRACER STUDIES WITH STRONTIUM-90

Marshall Parrott and J. G. Hamilton

Fig. 15 is the male Rhesus monkey, "Willie," born in our colony on July 7, 1954. The complete record of his weight and blood picture to date is shown in Table VII. Currently, his crown-to-rump height is 33.9 cm. The diet presently includes whole milk formula (2 grams sucrose per ounce milk), with banana flakes and Meritene protein supplement, plus small amounts of carrot, orange, chim biscuit, peanuts, and apple. He is continuing to receive supplementary vitamin and iron feedings. Since his birth the animal has been maintained apart from the rest of the colony in order that we might observe the behavior of a very young normal animal under the best conditions in anticipation of future experiments, and also because of the inability of the mother to lactate after the birth of "Willie." This inability to lactate necessitated a feeding formula on a two-hour, twenty-four-hour-day schedule by one or more members of the Laboratory for a period of about three weeks. His health has been generally good, though he has had an intestinal upset for the last month. The general health and blood picture of this animal indicate that there have been to date no untoward effects on growth and development as a result of his body burden at birth of approximately 0.17 microcuries of Sr^{90}.

On approximately May 12 another infant Rhesus monkey is due, the parents again being those of the above-mentioned animal. Disposition of this animal will be decided upon after its birth. The mother excreted 51.3% of the Sr^{90} within the first ten days after its administration. A weekly stool sample taken from the two hundred and twenty-first day indicated an excretion of 0.012% of the injected Sr^{90} per day. On the basis of these two measurements, the Sr^{90} body burden of the mother is currently estimated at 15.3 microcuries.



ZN-1272

Fig. 15. The male Rhesus monkey, "Willie," born in our colony on July 7, 1954.

Table VII

Blood count and body weight of male Rhesus monkey, "Willie" born July 7, 1954.

Date	Weight (kilograms)	White Blood Count	Red Blood Count	Hemoglobin
7/7/54	0.425	----	----	----
7/19/54	0.465	----	----	----
8/3/54	0.624	----	----	----
8/30/54	0.807	----	----	----
9/14/54	0.982	18,000	3,840,000	16.9
10/8/54	1.164	iron and penicillin started		
11/8/54	1.363	12,500	7,730,000	14.2
12/10/54	1.619	17,325	6,120,000	15.0
1/11/55	----	13,250	5,110,000	14.2
2/25/55	2.130	8,400	5,140,000	14.0
4/1/55	2.239	18,650	7,180,000	13.8

RADIATION CHEMISTRY

Warren M. Garrison in charge

INDIRECT ACTION OF RADIATION IN DILUTE FORMIC ACID SOLUTIONS

Winifred Bennett, Sibyl Cole, Michael Jayko, and Boyd M. Weeks

In the irradiation of dilute, oxygen-free formic acid solution by γ-radiation, it has been shown[1] that the net reaction may be represented by the equation

$$HCOOH \longrightarrow H_2 + CO_2, \qquad (1)$$

and that the mechanism may be interpreted in terms of the processes:

$$H_2O \xrightarrow{\sim\sim} H + OH, \qquad (2)$$

$$2H_2O \xrightarrow{\sim\sim} H_2 + H_2O_2, \qquad (3)$$

$$H + HCOOH \longrightarrow H_2 + COOH, \qquad (4)$$

$$OH + HCOOH \longrightarrow H_2O + COOH, \qquad (5)$$

$$2COOH \longrightarrow CO_2 + HCOOH, \qquad (6)$$

$$COOH + H_2O_2 \longrightarrow CO_2 + H_2O + OH, \qquad (7)$$

where 2 and 3 represent the primary radiation processes. In an earlier study,[2] it was found that as the pH of the formic acid is increased above 3, the production of both hydrogen and carbon dioxide decreases. The latter decreases more rapidly, and at pH 7 only hydrogen is obtained. It was susggested on the basis of the observed gas yields that both oxalic acid and formaldehyde may be formed in addition to hydrogen and carbon dioxide. Several years ago, in a preliminary report[3] from this laboratory, it was shown that irradiation of oxygen-free solutions of C^{14}-labeled formic acid results in the formation of formaldehyde, oxalic acid, and several other organic products which were at that time unidentified. In a recent continuation of these studies it was found[4, 5] that glycolic ($CH_2OHCOOH$), tartronic ($COOHCHOHCOOH$) and tartaric ($COOHCHOHCHOHCOOH$) acids are among the products synthesized in the irradiation of dilute aqueous formic acid solutions under conditions which result in the decomposition and transformation of less than 1% of the formic acid initially present in the target solution.

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[1] E. J. Hart, Radiation Res. 1, 53 (1954).
[2] H. Fricke, E. J. Hart, and H. P. Smith, J. Chem. Phys. 6, 229 (1938).
[3] W. M. Garrison, D. C. Morrison, H. R. Haymond, and J. G. Hamilton, J. Am. Chem. Soc. 74, 4216 (1952).
[4] Quarterly Report, UCRL-2605.
[5] Quarterly Report, UCRL-2823.

Hamilton

More recently, it has been established that glyoxylic (CHOCOOH) and mesoxalic (COOHCOCOOH) acids are also produced. Solutions containing C^{14}-labeled formic acid were irradiated to facilitate product identification by previously described methods of partition chromatography.[4,5] Coelution of authentic acid with the appropriate product activity is used as one procedure in establishing the identity of products. Coelution of glyoxylic acid with a C^{14}-labeled product formed in the 35-Mev helium-ion irradiation of 0.25 M HCOOH at a radiation dose of 10^{20} ev/cm^3 is shown in Fig. 16. The 2,4-dinitrophenylhydrazone derivative of the eluted glyoxylic acid showed a constant specific activity after repeated recrystallizations from methanol. Glyoxylic and mesoxalic acids were also indicated as products on the basis of filter paper chromatographic methods described in a later section.

The formation of higher aliphatic acids in irradiated formic acid solutions is most readily interpreted in terms of a pH effect on the relative rates of the disproportionation reaction 6 and a dimerization reaction 6a

$$2COOH \longrightarrow HCOOH + CO_2 \qquad (6)$$

$$2COOH \longrightarrow (COOH)_2 \qquad (6a)$$

The formation of glyoxylic and mesoxalic acids may then be attributed to a similar pair of competing reactions involving radicals formed by H atom reduction of oxalic acid, i.e.,

$$(COOH)_2 + H \longrightarrow C(OH)_2COOH \qquad (8)$$

$$COOH + C(OH)_2COOH \longrightarrow CO_2 + H_2O + CHOCOOH \qquad (9)$$

$$COOH + C(OH)_2COOH \longrightarrow H_2O + COOHCOCOOH \qquad (10)$$

The formation of formaldehyde indicates that the radicals HCO or $HC(OH)_2$ are also formed and these may be removed in the production of both formaldehyde and glyoxylic acid

$$CHO + COOH \longrightarrow CH_2O + CO_2 \qquad (11)$$

$$CHO + COOH \longrightarrow CHOCOOH \qquad (12)$$

The formation of glycolic and tartronic acids can be accounted for by a reaction of the type in Eq. (13),

$$CHOCOOH + H \longrightarrow CHOHCOOH, \qquad (13)$$

followed by Reactions (14) and (or) (15),

$$COOH + CHOHCOOH \longrightarrow CH_2OHCOOH + CO_2, \qquad (14)$$

$$COOH + CHOHCOOH \longrightarrow COOHCHOHCOOH. \qquad (15)$$

The most obvious mechanism for tartaric acid formation is

$$2CHOHCOOH \longrightarrow COOH(CHOH)_2COOH. \qquad (16)$$

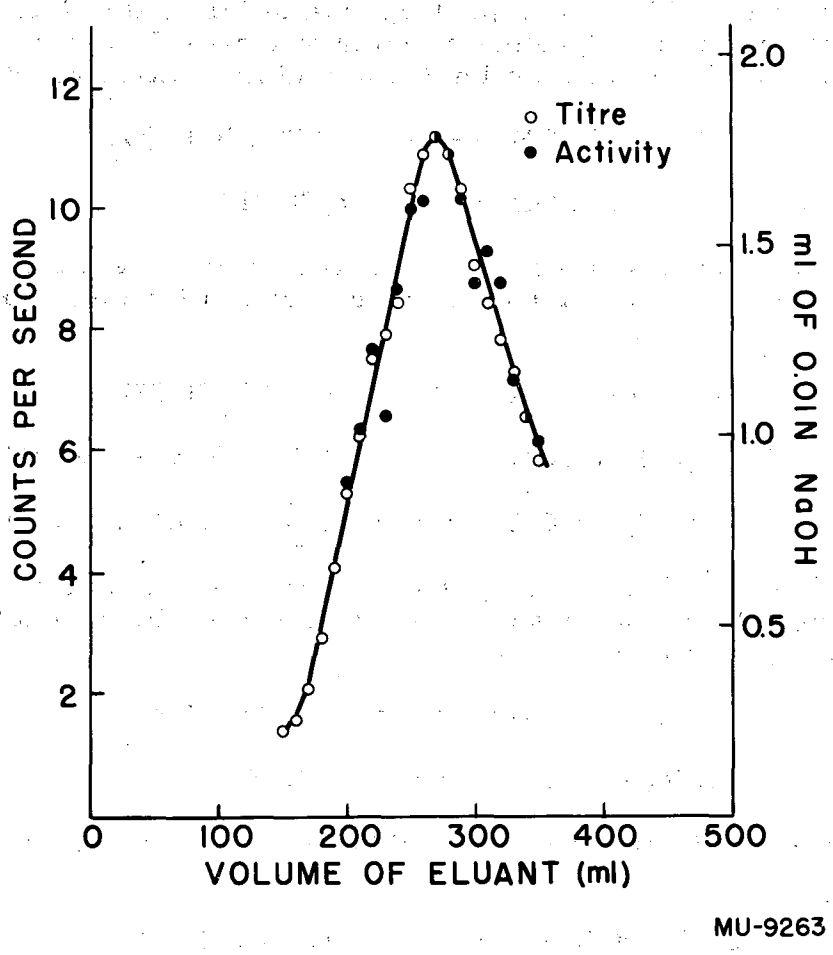

Fig. 16. Elution of glyoxylic acid with 10% butanol - 90% chloroform v/v saturated with 0.5 M sulfuric acid.

A detailed study of the mechanisms outlined above is now in progress. The effects of radiation dosage, pH, and concentration are being determined. Preliminary data on the effect of dosage and pH on the radiation yield of several products in 0.25 N formic acid are given in Table VIII. The solution containing added $HC^{14}OOH$ was aerated with helium during irradiation in the 10-ml all-glass target cell previously described.[4] After irradiation, platinum black and the appropriate carrier acids were added and the nonvolatile fraction was chromatographed.[4,5] Figures 17 and 18 show typical results obtained for oxalic and glycolic acid. Radiation yields were determined by the isotope dilution technique.

DEVELOPMENT OF ANALYTICAL METHODS FOR CARBONYL DETERMINATIONS

Separations on Powdered Cellulose Columns

Michael Jayko

Results obtained thus far have shown that several products are formed during the bombardment of aqueous formic acid by helium ions. Among these are a number of carbonyl compounds. As indicated earlier, the isolation and identification of all components are essential to an understanding of the mechanisms involved in the production of these compounds.

The separation of acetaldehyde, formaldehyde, and acetone by paper chromatography of their 2,4-dinitrophenylhydrazones, as reported in the Quarterly Report for April, May, June 1954, UCRL-2823, has been used as the basis for a new procedure for separation of milligram quantities of these materials. A column is prepared from a slurry of cellulose powder in methanol and then washed with a 15% solution of methanol in petroleum ether (bp 65°–110°). The percent of methanol is gradually decreased to 3%; this gradual decrease is necessary since a too rapid change in solvents at this point will cause the paper to pull away from the wall, forming channels. The compounds to be separated are added either in petroleum ether or 3% methanol in petroleum ether, using as small a volume of solvent as possible. The column is then developed with the latter solvent. The three compounds separate into distinct bands which can be collected in a small volume of solvent ether by extruding and extracting or by allowing them to pass out of the column.

The separation of the 2,4-dinitrophenylhydrazones of mesoxalic and glyoxylic acids has been accomplished using butanol saturated with 3% ammonia. This procedure could be transferred directly from a paper chromatogram to a cellulose powder column without changing the composition of the solvent. The mesoxalic and glyoxylic acid derivatives were found to have Rf values of 0.26 and 0.53 respectively. After a brief survey of the most promising solvents, it seemed unlikely that all five of the above-mentioned dinitrophenylhydrazone derivatives could be satisfactorily separated by using only one solvent. With a mixture, then, there was a choice of chromatographing with butanol-ammonia first and allowing the acetaldehyde, formaldehyde, and acetone to follow the front, or of using methanol–petroleum ether and having the mesoxalic and glyoxylic acids stay at the origin to be developed subsequently with butanol-ammonia. The latter procedure was found to be more desirable.

Hamilton

Table VIII

Radiation yields[a] of products from formic acid solutions
radiation: 35-Mev helium ions; beam current 0.2 μa; target volume, 10 ml.

	0.25 N HCOOH		0.25 N HCOONa	
	0.003 μahr	0.010 μahr	0.003 μahr	0.010 μahr
Product Acid				
Oxalic	0.13	0.061	1.02	0.49
Glycolic	0.05	0.039	(<0.05)[b]	(<0.05)[b]
Tartronic	0.17	0.13	0.11	0.13
Tartaric	0.057	0.036	0.011	0.017

[a]Radiation yields expressed as molecules of product formed per 100 ev absorbed energy.
[b]This value represents an upper limit.

Hamilton

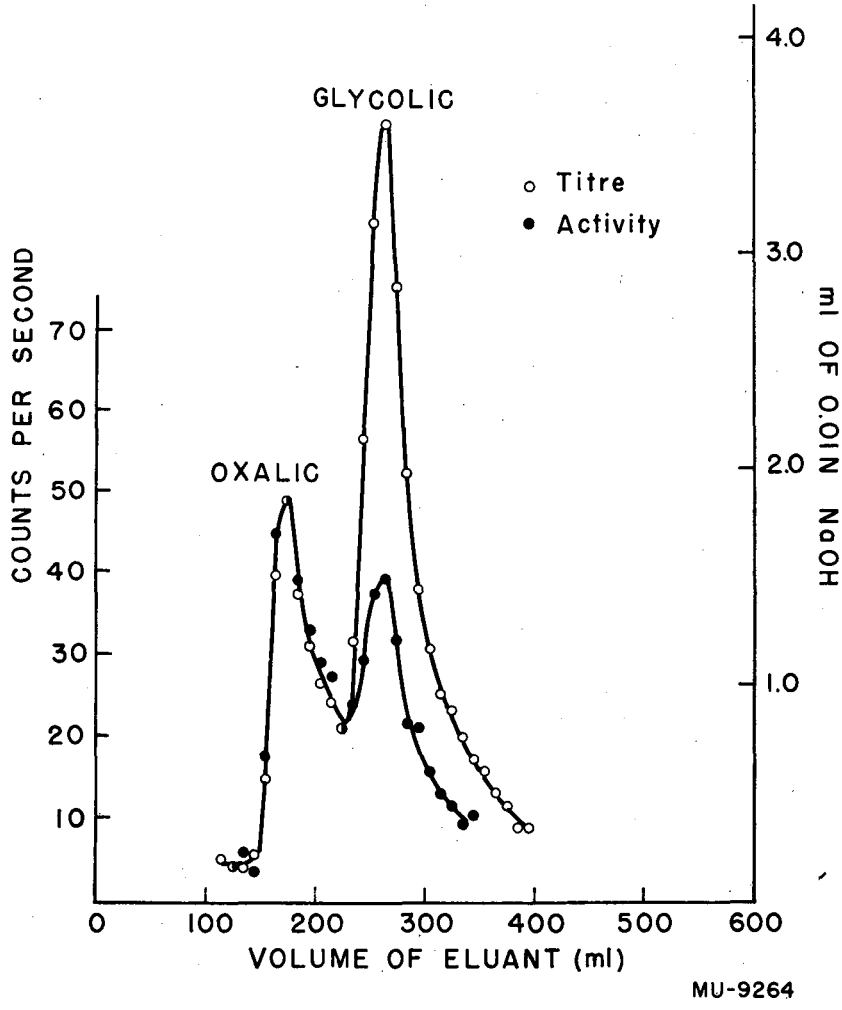

Fig. 17. Elution of glycolic and oxalic acids formed in 0.25 M formic acid solution containing $HC^{14}OOH$; eluant, 25% isobutanol – 75% chloroform v/v saturated with 0.5 M sulfuric acid.

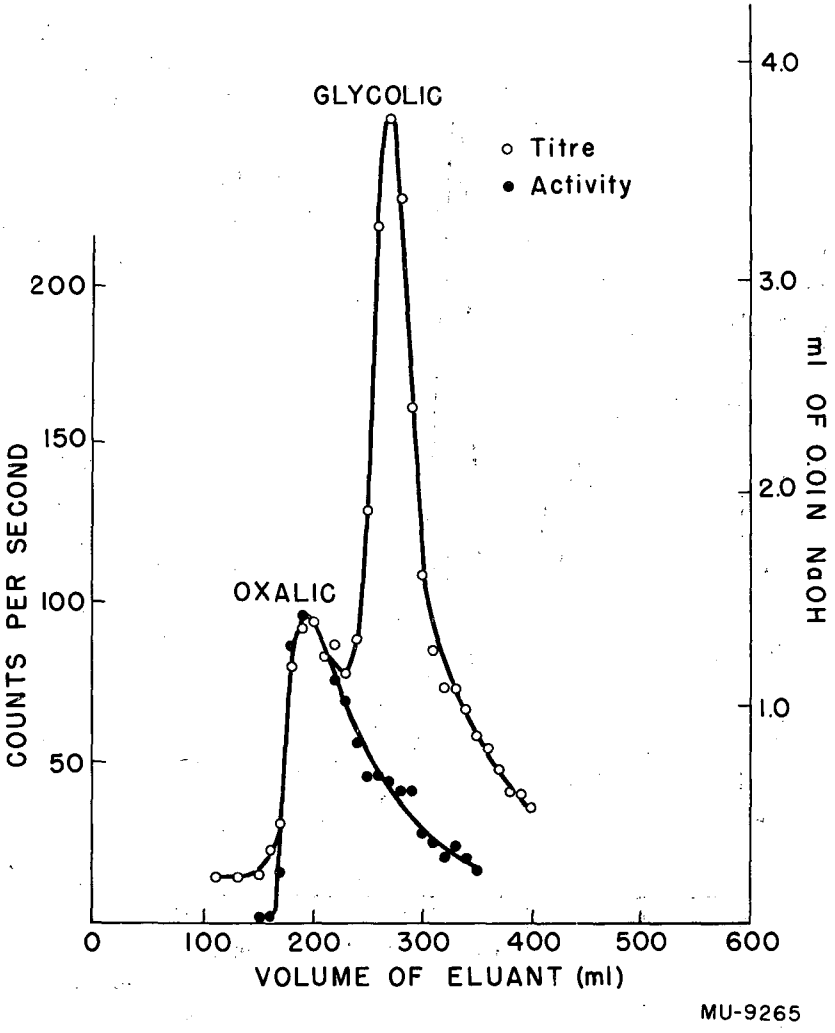

Fig. 18. Elution of glycolic and oxalic acids formed in 0.25 M sodium formate solution containing $HC^{14}OOH$; eluant as given for Fig. 2.

Quantitative Determination of Carbonyls

Winifred Bennett

Another extension of the qualitative chromatographic separations of 2,4-dinitrophenylhydrazone derivatives previously reported (Medical Physics Quarterly Report, July, August, September 1954, UCRL-2661), is the quantitative recovery of the purified materials from the paper. After the appropriate areas of the paper have been isolated, the desired compound can be extracted with a small volume of methanol in a spectrophotometer cell. Subsequent addition of a solution of 10% potassium hydroxide in 80% methanol-20% water, according to the method of Lappin and Clark, [1] produces an intensified color that can then be read colorimetrically to determine the concentration. However, because acetaldehyde and glyoxylic acid hydrazones show different fading characteristics, it will be necessary to obtain more detailed information on the behavior of these two derivatives.

GLYCINE

Boyd Weeks

Previous quarterly reports have described some of the methods and findings of a study of the action of 35-Mev helium ions (from the Crocker Laboratory 60-inch Cyclotron) on aqueous glycine solutions. A comprehensive summary and interpretation of the results of this study is now in preparation and will be offered as a UCRL report. A paper on this work, entitled "Indirect and Direct Action of Heavy-Particle Radiation on Glycine in Aqueous Solution," is also being prepared for presentation at the Radiation Research Society Meeting in May, 1955.

THE RADICAL PAIR YIELD FOR 35-MEV HELIUM IONS IN WATER

It has been shown that regardless of the type of high-energy radiation, the number of water molecules decomposed via the primary radiation processes

$$H_2O \sim\!\!\sim\!\!\sim\!\!\longrightarrow H + OH, \qquad (1)$$

$$2H_2O \sim\!\!\sim\!\!\sim\!\!\longrightarrow H_2 + H_2O_2 \qquad (2)$$

is 3.4 ± 0.3. With γ-rays and β-rays, most of the energy is utilized in the formation of H and OH via Reaction (1), whereas for α-rays, Reaction (2) is favored. Measurement of the radiation yield for water decomposition and relative contributions of (1) and (2) for cyclotron radiations is of importance in our studies for two reasons, namely, to provide an independent check of our beam monitoring and to establish whether or not it is possible at the high dose rates inherent in cyclotron irradiations to measure the total radical production via Reaction (1). We have accordingly initiated a comprehensive study

[1] G. R. Lappin and L. C. Clark, Anal. Chem. 23, 541 (1951).

of this problem. The formic acid–oxygen actinometer developed by Hart[1] is being employed. In this system the peroxide yield provides a measure of Reactions (1) and (2), and the yield of hydrogen is a measure of Reaction (2), i.e.,

$$OH + HCOOH \longrightarrow H_2O + COOH,$$

$$COOH + O_2 \longrightarrow CO_2 + HO_2,$$

$$H + O_2 \longrightarrow HO_2,$$

$$2HO_2 \longrightarrow H_2O_2 + O_2.$$

Preliminary results with 35-Mev helium ions at a beam intensity of 0.010 μa have been obtained. These data indicate that the radiation yeild G for hydrogen peroxide production in oxygenated 0.25 N formic acid solution is 2.4 ± 0.1. Effects of stirring, beam profile, dosage, and solute concentration are being evaluated.

REPORTS ISSUED

A summary of work done in this laboratory on the radiation chemistry of aquo-organic systems, and a discussion of the relationship of these studies to the more complex problems of radiation biology, have been prepared for the Geneva Conference; the Abstract of UCRL-2902 is included in this report. A paper entitled "Indirect and Direct Action of Heavy-Particle Radiation on Acetic Acid in Aqueous Solution," based on the summary report UCRL-2631, has been accepted for publication and will appear in the May 5 issue of the Journal of the American Chemical Society. A paper on the radiation chemistry of aqueous glycine solutions has been submitted for presentation at the forthcoming Radiation Research Society Meeting; an abstract is included in the glycine section of this Quarterly Report.

RADIATION CHEMISTRY OF AQUO-ORGANIC SYSTEMS

Warren M. Garrison

Elucidation of the processes of radiation biology is, in many instances, severely restricted by the difficulties involved in obtaining detailed information on the mechanisms of radiation-induced chemical change in biological systems. One basic approach to the interpretation of radiation biological phenomena, however, is the study of the mechanisms of indirect and direct action of radiation on simpler aquo-organic systems. It is from this viewpoint that a general investigation of the effects of cyclotron-produced radiation on organic compounds in aqueous solution was initiated in this Laboratory.

According to generally accepted concepts of the mechanism of indirect action on solutes at low concentration, the preliminary chemical effect of radiation absorption is the dissociation, by ionization and excitation, of water

[1] E. J. Hart, Radiation Res. 1, 53 (1954).

molecules to give free H and OH radicals. Reaction of H and OH with added organic solute results in the formation of secondary free radical species. The organic radicals so produced interact to form observed products through dimerization and (or) disproportionation. In the presence of an adequate concentration of dissolved oxygen, H atoms react preferentially with O_2 to form HO_2 which subsequently disproportionates to produce H_2O_2 and O_2. Reactions of OH with solute are not inhibited by oxygen. However, the organic radicals so formed react, in turn preferentially with oxygen to form: (1) the corresponding peroxy radical and (or) (2) HO_2 and a product molecule. Dimerization may be completely inhibited in the presence of oxygen. Detailed examples of the above processes of indirect action are presented in a discussion of the mechanisms involved in the radiation synthesis of (1) formic acid from carbonic acid (2) oxalic, glycolic, glyoxylic and tartronic acids from formic acid (3) succinic and tricarballylic acids from acetic acid and (4) glyoxylic, diamino succinic, amino succinic and succinic acids from glycine.

Although the mechanism of indirect action in dilute solution can be described in terms of intermediate organic radical production by H and OH attack on the solute, it becomes necessary, in considering radiation-induced processes in more concentrated solution, to evaluate the contribution of reactive intermediates formed by direct action of radiation on the organic solute molecule. For example, in dilute acetic acid solution, the principal products formed in oxygen-free systems by high-energy helium ion irradiation at doses below 5×10^{20} ev/ml are hydrogen, hydrogen peroxide, and succinic acid. The latter is produced through dimerization of CH_2COOH radicals formed by both H and OH reaction with acetic acid. Carbon dioxide, methane, ethane, and carbon monoxide are also formed in low yield. The radiation yields of all products increase with acetic acid concentration in the range 0.0625 to 1.0 M. With increasing acetic acid concentration above 1.0 M, a continuous decrease in radiation yields for hydrogen, hydrogen peroxide, and succinic acid is observed as a decreasing fraction of total incident radiation energy is absorbed in water. Radiation yields of carbon dioxide, methane, ethane, and carbon monoxide, however, increase continuously with acetic acid concentration and, with the exception of ethane, yield values for these products show a linear dependency on acetic acid concentration. It is also shown on the basis of a consideration of carbon dioxide, methane and ethane yields in the presence and absence of added substances which are effective in "quenching" radical reactions that (1) predissociation of excited acetic acid molecules is involved in the formation of gaseous products derived from acetic acid and that (2) the life of the excited state is sufficiently long that the predissociation may be assumed to occur outside the particle track. Analogous processes for formic acid-water and glycine-water systems are discussed.

BIOLOGICAL STUDIES OF RADIATION EFFECTS

John H. Lawrence, M. D., in charge

Material for this section is not at present available, but may be expected in the next Quarterly Report.

HEALTH CHEMISTRY

Nelson B. Garden

The moving of all chemistry work and personnel to the newly completed Bldg. 70 was effected during the present quarter; this operation consisted of the transfer of essentially all chemists and their equipment from Bldgs. 4, 5, and 50. Refinements of various aspects in the new quarters, such as ventilation, emergency power, etc., are being worked out, and numerous small details, including placement of personnel decontamination kits and covering of exposed cement with decontaminatable coatings, are receiving attention. The radioactive-chemicals storage areas and pit facilities are being completed, and a new ventilated cabinet for the storage of alpha-emitting samples was installed. The decontamination quarters now include a gloved hood setup for storing and decontaminating of gear too active for processing in gloved-box decontamination work but not worthy of the effort necessary in decontaminating it in the remote chambers.

Health Chemistry personnel were involved in the monitoring, selecting, packaging, discarding, and consigning to salvage of equipment and gear from the old quarters and its installation in the new throughout the moving operation.

By means of a 90-curie cobalt-60 source, gamma-ray photographs were made of the six-inch and two-inch caves in Bldg. 70 prior to subsequent completion and installation of cave accessories. Two voids of small magnitude were noted in the six-inch cave and radiation leaks were observed in the two-inch cave through nelson studs, lifting studs, and ball-socket manipulators.

The storage room in Bldg. 70 for active gloved box housing was modified so that those boxes containing emanation emitters were isolated from other storage items in order to prevent cross-contamination.

A small spill in the new building, involving transuranic emitters, again pointed out to the Chemistry personnel the necessity of adhering to Health Chemistry principles and of intelligent and mature use of equipment provided for protection. It also disclosed the mandatory aspect of decontaminatable coatings on floors and other surfaces.

Many man-hours were spent by Health Chemistry personnel in checking out the areas evacuated by the move to Bldg. 70. Building 50 was readied for occupancy by Physics personnel, and Bldg. 4 was prepared for outside contractors, who will reconstruct the interior of the building. The work on Bldg. 4 involved complete monitoring; marking of contaminated areas, including charted hidden contamination accumulated over the last eleven years (some of it from the Manhattan Project work); removal of contamination where possible, removal of linoleum, transite, and other surface materials; decontamination of hoods, checking of exhaust ducting and stacks; and removal and disposal of large quantities of sheet metal parts, pipes, and wood members. Contaminated sheet metal parts were crushed and put in coffins for sea disposal; piping that could be contaminated was sealed in cement in tanks for the same

fate, and wood that was decontaminated to a low number of counts underwent a special burning program on a remote spot on the Project area.

As Bldg. 4 is to be occupied by Health Chemistry after its revamping, designs have been submitted for the office quarters, engineering rooms, shops, hooded labs, etc. This plan will consummate a long-cherished desire, in that the entire Health Chemistry group--save for local stations in various Chemistry Department areas--will be housed under one roof, eliminating the present situation wherein administration and offices are in two different areas, gloved box and equipment development shops in another (on the Berkeley campus), shop facilities elsewhere, and routine airborne activities, control processes, and research studies in still another location.

New equipment designed and (or) installed by Health Chemistry during this period includes an oxygen-atmosphere sparking enclosure or chamber wherein a high-level curium specimen was processed with minimum hazard from sparking procedures. A californium sample irradiated at Idaho Falls MTR was processed in a two-inch-cave setup; this run disclosed the presence of higher gamma activity than had been anticipated, causing the suspicion of impurities in the quartz used for the capsule and leading to a program of testing of quartz for future use and stocking of selected, especially pure items.

A mockup of a new target holder for use in the external beam of the 60-inch cyclotron has been designed; the modifications of the new model may ultimately be applied to the pistol-grip type currently in use. A modification of the covering plate on the pistol-grip target holder was effected by perforating it, allowing recoil of bombarded atoms within the target chamber and ultimate deposition of these atoms on the back of the target; this step eliminated unwanted steps in the chemical processing of the target material.

Shock-mounted vacuum pumps have been provided for use in vacuum-line boxes for the purpose of protecting vibrating-sensitive instruments housed therein.

Designs are well in progress for the basic equipment and procedures for the six-inch cave in the new Bldg. 70. Those for carton transfer systems, dolly design, cave waste systems, and other process equipment are nearing completion.

Other work executed by the Equipment Development group includes preparation of small slugs and targets, repair of lead glass windows, repair of cave refrigeration, construction of special tongs and gadgets, fabrication of various envelopes of vinyl plastic, and drafting service for other Health Chemistry groups; this group has also instigated a formal training program for its members in order to best acquaint its people with problems relating to the whole field of radioactivity protection.

The Special Problems group continues to fabricate special sources of radioactive material on request, and approximately 50 such sources were made during this quarter. This group also continuously repackages the already existing sources, which are routinely checked by the monitoring group. The Laboratory's radium sources are also routinely checked for leakage, and those found to be faulty are repackaged for shipment to the National Bureau of Standards

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for repair or replacement. Standard corrosion and decontamination tests were run on four commercial products by this group, who found some promising materials. This group also assisted the Activity Handling group in the preparation of waste for sea disposal; it gave consultant assistance in the setting up of the neutralizing and gelling machines for the gelling of liquid waste; about 700 gallons of accumulated waste were processed during this quarter. A tentative flow sheet for the solidification of liquid waste by evaporation was drawn up.

Members of the Health Chemistry group have been engaged in off-Project work at the Nevade Test Site; as consultants at the Scripps Institution of Oceanography at La Jolla, California; at the California Public Health Service; and at the various University of California campuses throughout the State.

The Airborne Activity Control Group, in addition to its work in the transfer of equipment to Bldg. 70, the participation in the designs for new projects in Bldg. 70, and the demolition of Bldg. 4, acted as consultants in the collection of fall-out data at the Nevada Test Site, at the request of the Atomic Energy Commission.

A new job classification of Radiological Technologist has been created at the Radiation Laboratory, which classification effects satisfactorily the status of many Health Chemistry members.

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HEALTH PHYSICS

Burton J. Moyer

STATISTICAL SUMMARY OF MONITORING PROGRAM

Survey Instruments Maintained

β-Ionization Chamber	105
I. D. L. Portable Survey Instruments	23
Cutie Pies	3
Recording - Intensity Meters	32
Victoreen Proteximeter	3
Fast-Neutron Proportional Counters	8
Slow-Neutron Proportional Counters	15
Fast-Neutron Proportional Counter (Portable)	21
Slow-Neutron Portable Unit	16
Balanced Chamber--Fast-Neutron--Portable	3
Special Tissue Wall Survey Instrument	1

Personnel Meters in Use

Total Personnel Covered with Film Badges	3276
Total Man-Days Coverage with Pocket Chamber	9157
Total Man-Days Coverage with Pocket Dosimeters	9157
Total Man-Days Coverage with Pocket Chambers (S.N.)	8999

Cases of Weekly Exposure Above 0.3r

Weekly film expos. above	184" Area	60" Area	Linac	Chem.	Other	Total
0.3	0	16	3	18	5	42
0.5	0	4	1	4	0	9
1.0	0	1	0	0	0	1
1.5	0	0	0	0	0	0
2.0	0	0	0	0	0	0
2.5	0	0	0	0	0	0
3.0	0	0	0	0	0	0

Information Division
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