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EDITORS: H. H. ANDERSON, D. B. TAYLOR, E. L. WAY

Volume 2, No. 21, pp. 283–344, plates 10–26, 8 figures in text

Submitted by editors October 29, 1952
Issued February 27, 1953
Price, $1.25

UNIVERSITY OF CALIFORNIA PRESS
BERKELEY AND LOS ANGELES
CALIFORNIA



CAMBRIDGE UNIVERSITY PRESS
LONDON, ENGLAND

PRINTED IN THE UNITED STATES OF AMERICA

# THE ACCUMULATION, METABOLISM AND BIOLOGICAL EFFECTS OF ASTATINE IN RATS AND MONKEYS

BY

JOSEPH G. HAMILTON, C. WILLET ASLING, WARREN M. GARRISON, AND KENNETH G. SCOTT

(Contribution from the Division of Medical Physics, the Crocker Radiation Laboratory, and the Departments of Medicine, Anatomy, and Radiology of the University of California, Berkeley and San Francisco)[1]

## INTRODUCTION

In 1940 Corson, MacKenzie, and Segre isolated a radioactive element whose chemical, physical, and nuclear properties established it to be element 85, the last of the halogen group. This element, now named astatine (from the Greek ἄστατος, "unstable") was produced by the transmutation of bismuth by alpha particles accelerated to 30 Mev[2] in the 60-inch cyclotron at the Crocker Laboratory.

Hamilton and Soley (1940) briefly compared the metabolism of radio-iodine and astatine, and demonstrated that the manner of their accumulation in the thyroid gland was similar. They studied both normal guinea pigs and guinea pigs in which thyrotoxicosis had been produced by administration of the thyrotropic hormone. Changes in research programs necessitated by the defense effort unfortunately precluded further studies with astatine.

Recently Hamilton *et al.* (1950) presented a short report concerning the destructive action of astatine on the thyroid gland of the rat. They observed that relatively small amounts of astatine were capable of provoking profound changes without inducing any noticeable alterations of structure in the parathyroid gland or other peritrachial tissues. This observation was at variance with the finding of Goldberg *et al.* (1950), who accomplished comparable thyroid destruction by the use of radio-iodine. The difference presumably arose from the fact that the isotope employed, astatine$^{211}$ with a half-life of 7.5 hours, emits energetic alpha particles whose range is of the order of 70 microns in tissue, whereas the far less energetic beta particles emitted from radio-iodine have a maximum range of approximately 2,000 microns in tissue.

[1] This work was performed under contract No. W-7405-eng-48-A of the University of California under the United States Atomic Energy Commission.

[2] Million electron volts.

A comparison of the behavior of the thyroid gland toward other elements of group VII of the periodic table, such as chloride, bromide, and iodide, is pertinent. Baumann and Metzger (1949) presented evidence for a small preferential accumulation of chloride ion by the thyroid gland.

A number of investigators have shown that there is apparently a small but significant degree of selective localization of bromide ion by the thyroid gland (Baumann *et al.*, 1941; Perlman *et al.*, 1941; Simon, 1947; and Baumann, *et al.*, 1951). Simon showed that the bromine accumulated by the thyroid gland was apparently present exclusively as bromide ion. Davenport and Fisher (1940) demonstrated that bromide ion was secreted selectively by the stomach. This phenomenon was also observed in radio-iodine tracer experiments by Shiff *et al.* (1947) and Johnson and Albert (1951).

Similarity in chemical properties is to a great extent dependent on similarity in valence. The metabolic properties of many elements, other than those of group VII, of similar chemical properties and valence have been investigated (Hamilton, 1948).

The rapidity with which radio-iodine is bound by the normal thyroid gland into relatively stable organic compounds was first shown by Perlman *et al.* (1941). This observation has been confirmed by many investigators, including Fink and Fink (1948), Chaikoff and Taurog (1949), and Taurog *et al.* (1950). The radio-iodine bound in the thyroid gland is apparently contained in thyroglobulin, since relatively little can be dialyzed and almost all can be precipitated 12 to 24 hours after administration by the use of a half-saturated ammonium sulfate solution following homogenization of the gland (Scott, 1950).

The object of the experiments here presented was to study the accumulation of astatine in the thyroid gland, its metabolism and biologic action, and possible deleterious effects. It was considered mandatory to determine the effects on laboratory animals before studies in man were attempted. Rats and monkeys were selected as test subjects.

## TRACER STUDIES

By means of tracer studies, data were elicited concerning the accumulation of astatine by the thyroid gland of the rat; the concomitant accumulation of astatine and radio-iodine; the influence of premedication with stable iodine on astatine and radio-iodine accumulation; and the effect of an iodine-deficient diet on the accumulation of astatine. The studies with monkeys provided data on the accumulation of these radio-

elements by the thyroid gland. The metabolism of astatine and radio-iodine in the rat were compared, and astatine and astatine-radio-iodine radioautographs were prepared from thyroid sections.

## Methods

The rats were adult laboratory animals, maintained on standard laboratory chow and tap water for a month previously in an attempt to establish a state of iodine balance. The monkeys were young *Macacus rhesus,* weighing about 2.5 kilograms, maintained on a diet of fresh vegetables, bananas, peanuts, "Chim" crackers, and tap water. The iodine content of the food and water was not determined, as iodine was present and was frequently used in other rooms of the laboratory.

The radio-iodine contained less than 0.01 microgram of stable iodine per microcurie, and was given as sodium iodide[8] dissolved in "isotonic" sodium chloride, pH 7. The astatine solutions contained sodium sulfite at a concentration of 0.01 M as well as the sodium chloride to maintain a minus 1 valence state. This precaution was taken to preclude the possibility of volatilization, or plating out of astatine on the metallic surfaces of the needles employed for injection.

*Accumulation of astatine in rat thyroid.*—One hundred fifty-nine rats of both sexes were given from 5 to 50 microcuries of astatine intravenously, and sacrificed in groups from one to seventy-two hours later. Seventy of these rats had also received radio-iodine as part of the second experiment.

The astatine in the thyroid glands was assayed by counting the alpha particles as described in the Appendix.

*Concomitant accumulation of astatine and radio-iodine.*—The same time intervals were used for the second study, with twenty rats sacrificed at twenty-four hours and ten in each of the other groups. The solution containing both radio-iodine and astatine was given intravenously in doses of 5 microcuries of the former and from 5 to 50 microcuries of the latter. The short half-life of astatine made necessary the larger doses for the longer time intervals.

The radio-iodine accumulation was determined by counting its gamma rays with a Geiger counter, and the astatine content was measured by counting the alpha particles with the argon gas counter as explained in the Appendix.

*Influence of stable iodine.*—The first of four groups of rats received 1 milligram of potassium iodide and 20 microcuries of astatine simul-

[8] Stated analysis by the Isotope Division of the Oak Ridge National Laboratory, United States Atomic Energy Commission, Oak Ridge, Tennessee.

taneously by intravenous injection. The second group were given 1 milligram of stable potassium iodide by parenteral injection daily for six days. On the sixth day they also received astatine. The third group received astatine after two weeks on an iodine-deficient diet. The F-5 synthetic diet of the Institute of Experimental Biology (University of California, Berkeley) was used, without the customary iodine supplement. The fourth group were given 1 milligram of potassium iodide daily for six days, and on the sixth day they also received 5 microcuries of radio-iodine by intraperitoneal injection. Control rats were provided for each group.

All the test rats and their controls were sacrificed twenty-four hours after receiving astatine or radio-iodine, and the thyroid glands were assayed.

*Accumulation of astatine and radio-iodine in the monkey.*—Observations of limited scope were made on two monkeys to obtain radioautographs, and also to determine and compare the accumulation by the thyroid gland of the two radioelements.

The first monkey received 35 microcuries of astatine intraperitoneally; the second received 140 microcuries of astatine and 68 of radio-iodine. Both were sacrificed nineteen hours later.

*Metabolism of astatine and radio-iodine.* Female Long-Evans rats were maintained for one month on standard laboratory chow, with free access to tap water. When the astatine was given, they weighed from 130 to 170 grams. They were divided into five groups of three animals each. Those in the 1-, 4-, and 24-hour groups received 50 microcuries of astatine intravenously, and those in the 9- and 13-hour groups received 35 microcuries. These dose levels were possibly at the lower level of radiation injury, but circumstances of the experiments dictated the amounts to be used. Because of the relatively brief intervals of the entire experiment, it was considered unlikely that any significant aberration of the metabolic pattern of astatine would be encountered. Moreover, because of the short half-life of astatine, it was not feasible to extend the duration of the experiments, employ more animals, or work with more than one group at a time. The tissues and excreta were assayed by counting the polonium$^{211}$ x-rays produced by the decay of astatine$^{211}$ using the crystal scintillation counter.

The experiment was repeated, using 37 microcuries of radio-iodine instead of astatine. Other circumstances were almost identical, although the rats weighed slightly more—from 170 to 200 grams. The scintillation counter was used to assay radio-iodine by counting the gamma rays,

in order to secure the maximum degree of efficiency, since the various preparations were counted wet and not subjected to any chemical treatment. A brief description of this counter is given in the Appendix.

The rats were maintained in cages with fine mesh floors, below which were large porcelain dishes to collect the urine. When astatine was

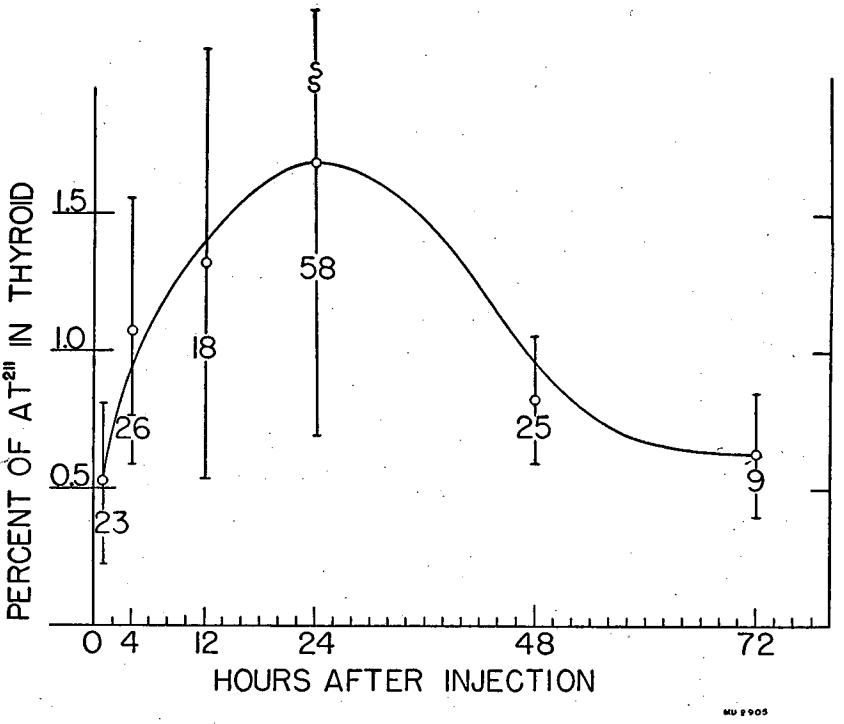


Fig. 1. The accumulation of astatine by the thyroid gland of the rat expressed as the per cent of the amount administered by intravenous injection. The range of uptake and number of animals used for each of the six time intervals are indicated.

used, both the screen and the dish were coated with paraffin to prevent plating out of the astatine, and sodium hydroxide and potassium iodide were added to each dish in such quantities that when the urine was diluted to a volume of 250 cubic centimeters at the time of assay, the concentration would be 1.0 M of sodium hydroxide and 0.5 M of potassium iodide. These precautions were unnecessary when radio-iodine was used.

When individual tissues weighed from 200 milligrams to 10 grams, it was possible to assay the entire specimen. However, some of the organs

and tissues were pooled, rather than individually assayed, for the three animals in each group. Pooled tissues included brain, pancreas, lymph nodes, ovary, and adrenal, pituitary, lacrimal, and salivary glands. The assay of large organs, notably skin, blood, muscle, and skeleton, required specimens weighing from 2 to 10 grams. For skin assay, the entire pelt

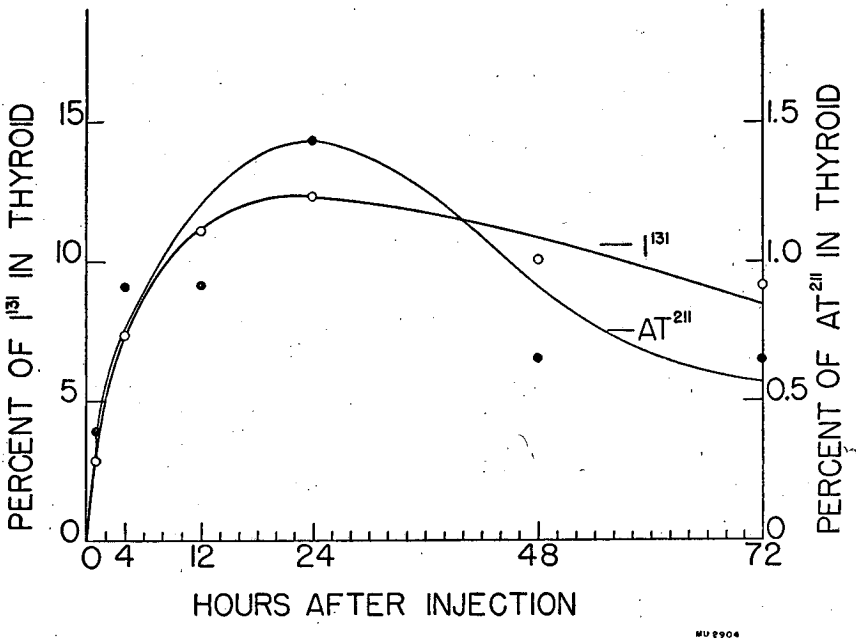


Fig. 2. A direct comparison of the accumulation of astatine and radio-iodine by the thyroid gland of the rat. Each animal received both radiohalogens by intravenous injection. The uptake of astatine is indicated on the right, and the uptake of radio-iodine on the left.

was removed and samples taken. Seven per cent of the total body weight was assumed to be the mass of the blood, and the value of the hematocrit was assumed to be 45 per cent. The introduction of this factor was necessary, since the blood, which was heparinized, was separated into plasma and cells. The total mass of muscle was assumed to be 45 per cent of the body weight, and that of the skeleton, 8 per cent of the body weight.

After tissues and organs had been removed, the skinned carcass was run through a meat grinder and a sample weighing from 2 to 10 grams was taken for assay. The calculated values for the amount of radio-element present in muscle, skeleton, and blood was subtracted from the

content of the entire carcass. Thus an attempt was made to estimate the amount present in residual biologic materials such as extracellular fluid, connective tissue, fat, glandular tissues, lymphoid tissue, blood vessels, teeth, spinal cord, and peripheral nerves (the "balance").

*Radioautographs.*—Astatine radioautographs were prepared from rat thyroid tissue. Each rat received 50 microcuries of astatine intravenously and was sacrificed eighteen hours later.

Since the alpha particles from astatine produce characteristic tracks in a photographic emulsion, whereas the beta particles from radio-iodine produce small grains of silver, an attempt was made to prepare a dual radioautograph. Thyroid tissue was taken from the second monkey, which had received both radioelements. The details of radioautographic and histologic techniques are presented in the Appendix.

## Results

*Accumulation of astatine in rat thyroid.*—The uptake of astatine by the thyroid gland of the rat is shown in figure 1, where it is expressed as the per cent of the administered dose. In this figure, the number of animals used at each time interval is shown, as well as the spread of accumulation at each point. The time of maximum accumulation seemed to be 24 hours; thereafter considerable loss occurred.

*Concomitant accumulation of astatine and radio-iodine.*—Figure 2 shows the rates of thyroid accumulation of the radioelements when given to the rat simultaneously. The uptake was rapid and similar, and a relatively greater loss of astatine seemed evident from these data. The accumulation of astatine was approximately one-tenth that of radio-iodine for each of the six time intervals.

*Influence of stable iodine.*—The data shown in table 1 present information that is in part apparently quite conclusive and in part suggestive that the amount of iodine present in the body does influence the accumulation of astatine and radio-iodine by the thyroid gland. The particular experiment in which stable iodine was given for six consecutive days demonstrated quite a marked effect in the reduction of the astatine accumulated by the thyroid gland. The effect of a single dose of 1 milligram of potassium iodide was less striking, as would be expected. There was far greater inhibition of radio-iodine than of astatine accumulation following the administration of stable iodine. The feeding of an iodine-deficient diet gave less definitive results. There was apparently, however, some enhancement of the capacity of the thyroid gland to accumulate astatine.

*Accumulation of astatine and radio-iodine in the monkey.*—The uptake of astatine by the thyroid gland of the first monkey was 20.7 per cent of the administered dose nineteen hours after injection. The second animal showed a thyroid accumulation of 9.47 per cent of astatine and 63.6 per cent of radio-iodine.

TABLE 1

THE EFFECT OF THE ADMINISTRATION OF STABLE IODINE AND A LOW-IODINE DIET UPON THE UPTAKE OF ASTATINE BY THE THYROID GLAND OF THE RAT

(The uptake of radio-iodine by the thyroid gland after administration of stable iodine is included for comparison.)

| | Per cent in thyroid gland | Range | Number of rats |
|---|---|---|---|
| 1 Mg KI given with At | | | |
| Control | 1.74 | 1.00–2.46 | 16 |
| Treated | 0.45 | 0.37–0.53 | 5 |
| 1 Mg KI daily x6 followed by At | | | |
| Control | 0.84 | 0.57–1.11 | 5 |
| Treated | 0.10 | 0.06–0.14 | 10 |
| Low-iodine diet before At | | | |
| Control | 0.97 | 0.46–1.48 | 5 |
| Treated | 2.43 | 1.35–3.51 | 10 |
| 1 Mg KI daily x6 followed by $I^{131}$ | | | |
| Control | 18.9 | 14.9–25.2 | 5 |
| Treated | 0.42 | 0.27–0.60 | 5 |

*Metabolism of astatine and radio-iodine.*—A comparison of the metabolism of the two radioelements in the rat is presented in tables 2 through 5. The per cent per gram accumulation of both was far greater in the thyroid gland than in any other tissue, and the greater concentration of radio-iodine in this organ is apparent.

A significant observation was that astatine usually showed a higher initial concentration per gram in other tissues and, at the later time intervals, disappeared more slowly than radio-iodine. There was more astatine in the tissues than in the plasma, with the exception of muscle at one hour and brain at all five time intervals. Conversely the radio-iodine content of plasma was greater than in most tissues at all time intervals. The urinary excretion of astatine was much slower. The values for fecal excretion suggest that relatively more astatine was eliminated

by this route, but the possibility of contamination of feces with urine cannot be excluded.

A noteworthy observation is the high values for astatine in stomach and intestinal contents. This effect also occurred with radio-iodine but was less prolonged. The concentration of astatine in the spleen, lung, ovary, pituitary, and adrenal gland, although less than in the stomach, was greater than in most other tissues at all time intervals. The one-hour values for the pituitary and lacrimal glands are not so significant, since these were single observations of pooled tissue.

A cursory inspection of the data would suggest that astatine is firmly bound in most of the tissues. If the per cent per gram of the tissue content of astatine is divided by the per cent per gram in plasma, however, an interesting degree of constancy may be noted for most of the tissue at all five time intervals. The corresponding values for radio-iodine have also been computed, and both are presented in table 4.

In an attempt to secure additional information from this material, the ratio between the astatine and radio-iodine values was calculated from table 4. The results of these computations are given in table 5. High values may be noted for spleen, adrenal gland, lymph node, lung, and ovary. All the values are greater than one with the exception of the thyroid gland, which fell to a level of 0.06 at twenty-four hours. The purpose of these computations and their interpretations appear in the discussion.

*Radioautographs.*—A representative astatine radioautograph from the thyroid gland of a rat is shown in plate 10. The presence of astatine is indicated by the straight black lines, which are the images produced in the photographic emulsion by the alpha particles. Because some leaching takes place during fixation, the question could be raised as to the validity of the radioautographs in representing the site of deposition of astatine at the time of sacrifice. It may be noted that there is a considerable variation in the number of tracks in the different acini. Any marked leaching of presumably bound astatine would have tended to make the distribution uniform rather than nonuniform. Another observation was that alpha particles were abundant in thyroid tissue but were rarely observed in the parathyroid gland and other adjacent tissues. In general, the deposition of astatine in the thyroid gland of the rat tended to be greater in the smaller acini more centrally located. The larger acini, which are more often present about the periphery, frequently accumulated less.

A photomicrograph of a dual astatine and radio-iodine radioautograph from the thyroid gland of the second monkey is shown in plate 11.

TABLE 2

THE DISTRIBUTION OF $At^{211}$ IN THE RAT 1, 4, 9, 13, AND 24 HOURS AFTER INTRAVENOUS INJECTION

(Values expressed in per cent of administered dose. The animals sacrificed at 1, 4, and 24 hours received 50 microcuries of $At^{211}$. The 9- and 13-hour animals received 35 microcuries of $At^{211}$.)

| Organ | 1 hour | | 4 hours | | 9 hours | | 13 hours | | 24 hours | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Per cent per organ | Per cent per gram | Per cent per organ | Per cent per gram | Per cent per organ | Per cent per gram | Per cent per organ | Per cent per gram | Per cent per organ | Per cent per gram |
| Heart | 0.68 | 0.86 | 0.37 | 0.54 | 0.34 | 0.43 | 0.33 | 0.46 | 0.38 | 0.62 |
| Muscle | 17.4 | 0.23 | 14.9 | 0.22 | 9.70 | 0.16 | 10.5 | 0.17 | 10.8 | 0.17 |
| Skeleton | 8.73 | 0.66 | 6.36 | 0.53 | 3.73 | 0.34 | 4.28 | 0.38 | 4.40 | 0.38 |
| Skin | 19.6 | 0.80 | 17.0 | 0.74 | 15.5 | 0.68 | 17.7 | 0.79 | 14.7 | 0.70 |
| Cells (blood) | 2.12 | 0.37 | 1.74 | 0.33 | 0.98 | 0.20 | 1.17 | 0.24 | 1.22 | 0.24 |
| Plasma | 1.51 | 0.26 | 1.11 | 0.21 | 0.66 | 0.14 | 0.65 | 0.13 | 0.86 | 0.17 |
| Brain | 0.22 | 0.14 | 0.13 | 0.081 | 0.11 | 0.07 | 0.095 | 0.065 | 0.11 | 0.066 |
| Eyes | 0.11 | 0.38 | 0.083 | 0.30 | 0.053 | 0.20 | 0.043 | 0.17 | 0.070 | 0.26 |
| Kidney | 1.56 | 1.12 | 1.37 | 0.95 | 1.02 | 0.66 | 0.98 | 0.65 | 0.95 | 0.78 |
| Liver | 5.41 | 0.77 | 3.68 | 0.63 | 6.09 | 0.71 | 5.71 | 0.74 | 2.96 | 0.45 |
| Spleen | 3.19 | 3.08 | 2.69 | 2.78 | 1.81 | 1.65 | 1.18 | 1.31 | 1.40 | 2.05 |
| Lymph node | ..... | 0.53 | ..... | 1.10 | ..... | 1.00 | ..... | 0.76 | ..... | 0.85 |
| Lung | 3.31 | 2.52 | 2.83 | 2.43 | 2.20 | 1.85 | 2.45 | 1.98 | 2.29 | 2.15 |
| Stomach | 5.19 | 3.87 | 9.90 | 8.29 | 7.42 | 6.04 | 8.19 | 6.70 | 5.85 | 5.68 |
| Small intestine | 4.26 | 0.99 | 2.64 | 0.79 | 2.96 | 0.74 | 3.06 | 0.85 | 2.14 | 0.85 |
| Caecum | 1.22 | 0.79 | 0.42 | 0.54 | 0.37 | 0.43 | 0.44 | 0.43 | 0.78 | 0.93 |
| Large intestine | 1.41 | 1.05 | 0.78 | 0.64 | 1.09 | 0.70 | 0.96 | 0.65 | 0.90 | 0.75 |
| G. I. contents | 8.82 | ..... | 12.7 | ..... | 9.96 | ..... | 7.86 | ..... | 10.5 | ..... |
| Pituitary | 0.077 | 10.0 | 0.018 | 2.48 | 0.008 | 1.25 | 0.009 | 1.13 | 0.009 | 1.00 |
| Adrenal | 0.078 | 1.62 | 0.072 | 1.41 | 0.048 | 0.84 | 0.052 | 0.72 | 0.039 | 0.78 |
| Pancreas | 0.64 | 0.83 | 0.46 | 0.57 | 0.25 | 0.40 | 0.20 | 0.36 | 0.41 | 0.49 |
| Ovary | 0.14 | 1.60 | 0.13 | 1.39 | 0.071 | 0.98 | 0.068 | 0.84 | 0.076 | 1.17 |
| Thyroid | 1.10 | 58.9 | 1.87 | 115.0 | 1.59 | 90.1 | 2.48 | 154.0 | 2.65 | 151.0 |
| Lacrimal gland | ..... | 4.32 | ..... | 0.68 | ..... | 0.49 | ..... | 0.33 | ..... | 0.67 |
| Salivary gland | 0.087 | 0.68 | 0.089 | 0.76 | 0.077 | 0.53 | 0.085 | 0.38 | 0.058 | 0.54 |
| Balance | 16.5 | ..... | 15.0 | ..... | 7.92 | ..... | 7.17 | ..... | 4.10 | ..... |
| Urine | 0.83 | ..... | 7.66 | ..... | 15.1 | ..... | 17.7 | ..... | 14.0 | ..... |
| Feces | 0.03 | ..... | 0.41 | ..... | 1.55 | ..... | 2.12 | ..... | 3.00 | ..... |
| Recovery | 104.3 | ..... | 104.4 | ..... | 90.6 | ..... | 95.5 | ..... | 84.7 | ..... |

TABLE 3

THE DISTRIBUTION OF $I^{131}$ IN THE RAT 1, 4, 9, 13, AND 24 HOURS AFTER INTRAVENOUS INJECTION

(Values are expressed in per cent of administered dose. All the animals received 37 microcuries of $I^{131}$.)

| Organ | 1 hour | | 4 hours | | 9 hours | | 13 hours | | 24 hours | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Per cent per organ | Per cent per gram | Per cent per organ | Per cent per gram | Per cent per organ | Per cent per gram | Per cent per organ | Per cent per gram | Per cent per organ | Per cent per gram |
| Heart | 0.17 | 0.25 | 0.14 | 0.17 | 0.073 | 0.10 | 0.07 | 0.10 | 0.032 | 0.044 |
| Muscle | 10.2 | 0.13 | 8.09 | 0.092 | 4.17 | 0.049 | 4.03 | 0.052 | 1.38 | 0.019 |
| Skeleton | 3.19 | 0.26 | 2.42 | 0.18 | 1.17 | 0.091 | 1.09 | 0.091 | 0.42 | 0.038 |
| Skin | 21.6 | 0.72 | 14.6 | 0.45 | 9.33 | 0.31 | 7.94 | 0.26 | 3.76 | 0.14 |
| Cells (blood) | 2.67 | 0.41 | 2.28 | 0.33 | 1.37 | 0.19 | 0.99 | 0.16 | 0.31 | 0.055 |
| Plasma | 3.88 | 0.62 | 2.97 | 0.43 | 1.54 | 0.23 | 1.62 | 0.26 | 0.63 | 0.11 |
| Brain | 0.05 | 0.034 | 0.037 | 0.021 | 0.022 | 0.012 | 0.02 | 0.012 | 0.014 | 0.010 |
| Eyes | 0.043 | 0.13 | 0.027 | 0.10 | 0.015 | 0.049 | 0.012 | 0.039 | 0.010 | 0.026 |
| Kidney | 0.47 | 0.37 | 0.42 | 0.27 | 0.23 | 0.15 | 0.24 | 0.17 | 0.091 | 0.071 |
| Liver | 2.27 | 0.30 | 1.89 | 0.20 | 1.05 | 0.14 | 1.02 | 0.14 | 0.57 | 0.093 |
| Spleen | 0.18 | 0.23 | 0.18 | 0.17 | 0.088 | 0.093 | 0.078 | 0.094 | 0.032 | 0.035 |
| Lymph node | ..... | 0.17 | ..... | 0.12 | ..... | 0.071 | ..... | 0.063 | ..... | 0.032 |
| Lung | 0.57 | 0.51 | 0.39 | 0.29 | 0.26 | 0.17 | 0.22 | 0.17 | 0.074 | 0.065 |
| Stomach | 6.11 | 6.01 | 3.73 | 3.30 | 2.24 | 1.95 | 1.56 | 1.42 | 0.45 | 0.46 |
| Small intestine | 3.05 | 0.69 | 2.41 | 0.43 | 1.33 | 0.27 | 0.80 | 0.19 | 0.31 | 0.082 |
| Caecum | 0.20 | 0.30 | 0.16 | 0.25 | 0.19 | 0.21 | 0.30 | 0.32 | 0.059 | 0.089 |
| Large intestine | 0.35 | 0.28 | 0.29 | 0.19 | 0.19 | 0.13 | 0.22 | 0.17 | 0.066 | 0.057 |
| G. I. contents | 14.1 | ..... | 12.7 | ..... | 4.40 | ..... | 2.38 | ..... | 2.18 | ..... |
| Pituitary | <0.005 | <0.5 | <0.005 | <0.5 | <0.005 | <0.5 | <0.005 | <0.5 | <0.005 | <0.5 |
| Adrenal | 0.012 | 0.15 | 0.010 | 0.10 | 0.006 | 0.065 | <0.005 | <0.05 | <0.005 | <0.05 |
| Pancreas | 0.25 | 0.33 | 0.13 | 0.16 | 0.06 | 0.082 | 0.037 | 0.087 | 0.015 | 0.036 |
| Ovary | 0.033 | 0.31 | 0.023 | 0.18 | 0.014 | 0.10 | 0.013 | 0.11 | 0.005 | 0.038 |
| Thyroid | 5.55 | 274.0 | 10.1 | 484.0 | 23.6 | 1220.0 | 18.2 | 1010.0 | 28.0 | 1630.0 |
| Lacrimal gland | ..... | 0.18 | ..... | 0.17 | ..... | 0.081 | ..... | 0.094 | ..... | 0.032 |
| Salivary gland | 0.080 | 0.43 | 0.034 | 0.16 | 0.020 | 0.09 | 0.024 | 0.11 | 0.010 | 0.046 |
| Balance | 10.7 | ..... | 9.85 | ..... | 7.54 | ..... | 4.63 | ..... | 3.11 | ..... |
| Urine | 11.6 | ..... | 29.3 | ..... | 33.7 | ..... | 39.3 | ..... | 53.7 | ..... |
| Feces | 0.30 | ..... | 1.25 | ..... | 0.69 | ..... | 0.75 | ..... | 1.10 | ..... |
| Recovery | 97.6 | ..... | 103.5 | ..... | 93.3 | ..... | 85.6 | ..... | 96.3 | ..... |

TABLE 4

THE RELATIVE CONTENT OF ASTATINE AND RADIO-IODINE IN THE TISSUES OF THE RAT, COMPARED TO THEIR CONCENTRATION IN THE PLASMA

(The upper number is the value for astatine and the lower number is the value for radio-iodine.)

| Organ | 1 hour | 4 hours | 9 hours | 13 hours | 24 hours |
|---|---|---|---|---|---|
| Thyroid | 226.5 | 548.0 | 644.0 | 1185.0 | 888.0 |
| | *442.0* | *1,126.0* | *5,304.0* | *3,884.0* | *14,820.0* |
| Stomach | 14.9 | 39.5 | 43.1 | 51.5 | 33.4 |
| | *9.69* | *7.67* | *8.48* | *5.46* | *4.18* |
| Lung | 9.69 | 11.6 | 13.2 | 15.2 | 12.6 |
| | *0.82* | *0.67* | *0.74* | *0.65* | *0.59* |
| Spleen | 11.8 | 13.2 | 11.8 | 10.1 | 12.1 |
| | *0.37* | *0.40* | *0.40* | *0.36* | *0.32* |
| Ovary | 6.15 | 6.62 | 7.00 | 6.46 | 6.88 |
| | *0.50* | *0.42* | *0.43* | *0.42* | *0.35* |
| Adrenal | 6.23 | 6.71 | 6.00 | 5.54 | 4.59 |
| | *0.24* | *0.23* | *0.28* | *< 0.19* | *<0.45* |
| Lymph node | 2.04 | 5.24 | 7.14 | 5.85 | 5.00 |
| | *0.27* | *0.28* | *0.31* | *0.24* | *0.29* |
| Small intestine | 3.81 | 3.76 | 5.29 | 6.54 | 5.00 |
| | *1.11* | *1.00* | *1.17* | *0.73* | *0.75* |
| Kidney | 4.31 | 4.52 | 4.71 | 5.00 | 4.59 |
| | *0.60* | *0.63* | *0.65* | *0.65* | *0.65* |
| Skin | 3.08 | 3.52 | 4.86 | 6.08 | 4.12 |
| | *1.16* | *1.05* | *1.35* | *1.00* | *1.27* |
| Large intestine | 4.03 | 3.05 | 5.00 | 5.00 | 4.41 |
| | *0.45* | *0.44* | *0.57* | *0.65* | *0.52* |
| Liver | 2.96 | 3.00 | 5.07 | 5.69 | 2.65 |
| | *0.48* | *0.47* | *0.61* | *0.54* | *0.85* |
| Caecum | 3.04 | 2.57 | 3.07 | 3.31 | 5.47 |
| | *0.48* | *0.58* | *0.91* | *1.23* | *0.81* |
| Lacrimal gland | 16.6 | 3.24 | 3.50 | 2.54 | 3.94 |
| | *0.29* | *0.40* | *0.35* | *0.36* | *0.29* |
| Heart | 3.31 | 2.57 | 3.07 | 3.54 | 3.65 |
| | *0.40* | *0.40* | *0.43* | *0.38* | *0.40* |
| Salivary gland | 2.62 | 3.62 | 3.79 | 2.92 | 3.18 |
| | *0.69* | *0.37* | *0.39* | *0.42* | *0.42* |
| Pancreas | 3.19 | 2.71 | 2.86 | 2.77 | 2.88 |
| | *0.53* | *0.37* | *0.36* | *0.33* | *0.33* |
| Skeleton | 2.59 | 2.52 | 2.43 | 2.92 | 2.24 |
| | *0.42* | *0.42* | *0.40* | *0.35* | *0.35* |
| Cells (blood) | 1.42 | 1.57 | 1.43 | 1.85 | 1.41 |
| | *0.66* | *0.77* | *0.83* | *0.62* | *0.50* |
| Eyes | 1.46 | 1.43 | 1.43 | 1.31 | 1.53 |
| | *0.21* | *0.23* | *0.21* | *0.15* | *0.24* |
| Muscle | 0.88 | 1.05 | 1.14 | 1.31 | 1.00 |
| | *0.21* | *0.21* | *0.21* | *0.20* | *0.17* |
| Plasma | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | *1.00* | *1.00* | *1.00* | *1.00* | *1.00* |
| Brain | 0.54 | 0.39 | 0.50 | 0.50 | 0.39 |
| | *0.055* | *0.049* | *0.052* | *0.046* | *0.091* |

TABLE 5

THE RELATIVE SELECTIVITY (S) OF TISSUES OF THE RAT FOR ASTATINE AS COMPARED TO RADIO-IODINE

$$S = \frac{\frac{\%\ per\ gm}{plasma}\ (At^{211})}{\frac{\%\ per\ gm}{plasma}\ (I^{131})}$$

| Organ | 1 hour | 4 hours | 9 hours | 13 hours | 24 hours |
|---|---|---|---|---|---|
| Spleen | 31.9 | 33.0 | 29.5 | 28.1 | 37.8 |
| Adrenal | 26.0 | 29.2 | 21.4 | >29.2 | >10.2 |
| Lymph node | 7.56 | 18.7 | 23.0 | 24.4 | 17.2 |
| Lung | 11.8 | 17.3 | 17.8 | 23.4 | 21.4 |
| Ovary | 12.3 | 15.8 | 16.3 | 15.4 | 19.7 |
| Lacrimal gland | 57.2 | 8.10 | 10.0 | 7.06 | 13.6 |
| Large intestine | 8.96 | 6.93 | 8.77 | 7.69 | 8.48 |
| Brain | 9.82 | 7.96 | 9.62 | 10.9 | 4.29 |
| Salivary gland | 3.80 | 9.78 | 9.72 | 6.95 | 7.57 |
| Heart | 8.28 | 6.43 | 7.14 | 9.32 | 9.13 |
| Kidney | 7.18 | 7.18 | 7.25 | 7.69 | 7.06 |
| Pancreas | 6.02 | 7.32 | 7.94 | 8.39 | 8.73 |
| Liver | 6.17 | 6.38 | 8.31 | 10.5 | 3.12 |
| Eyes | 6.95 | 6.22 | 6.81 | 8.73 | 6.38 |
| Skeleton | 6.05 | 6.00 | 6.08 | 8.34 | 6.40 |
| Stomach | 1.54 | 5.15 | 5.08 | 9.43 | 7.99 |
| Small intestine | 3.43 | 3.76 | 4.52 | 8.96 | 6.67 |
| Muscle | 4.19 | 5.00 | 5.43 | 6.55 | 5.88 |
| Caecum | 6.33 | 4.43 | 3.37 | 2.69 | 6.75 |
| Skin | 2.66 | 3.35 | 3.60 | 6.08 | 3.24 |
| Cells (blood) | 2.15 | 2.04 | 1.72 | 2.98 | 2.82 |
| Thyroid | 0.51 | 0.49 | 0.12 | 0.31 | 0.06 |
| Plasma | .... | .... | .... | ..... | .... |

## THE BIOLOGICAL EFFECTS OF ASTATINE

Data were obtained on the effect of astatine on the thyroid gland in correlation with dosage, by determining its subsequent ability to accumulate radio-iodine, by radioautographs, and by histopathologic changes.

Consideration of the tracer data made it clear that the action of astatine on structures of the body other than the thyroid gland should be investigated if this radioelement is ever to be considered for use in

man. The general physical effects resulting from the radiotoxicity of astatine were evaluated, and the histologic changes in other tissues were studied. A limited study was made of the effects of astatine on the monkey.

## Methods

The test animals were white Slonaker female rats, weighing from 180 to 200 grams, and *Macacus rhesus* monkeys. The rats and monkeys were maintained as for the tracer studies.

Seven groups of five rats each were given intravenously 0.5, 1.0, 10.0, 50.0, 70.0, 100.0, and 150.0 microcuries of astatine respectively. An eighth group served as control and was given "isotonic saline" solution intravenously instead of astatine. Each animal in the eight groups received 5 microcuries of radio-iodine intraperitoneally forty days after the administration of astatine; all were sacrificed twenty-four hours later, and the thyroid gland with a part of adjacent trachea was removed.

Determinations of the red cell count and total lymphocyte, leukocyte, and neutrophile counts were done for the 50-, 100-, and 150-microcurie dose levels at approximately five-day intervals for forty days after the administration of astatine. Body weights were determined for these three groups and for the control group at comparable time intervals. It was not possible adequately to follow the blood counts of the other groups and the control animals.

*Effect on the thyroid gland.*—The ability of the thyroid gland to accumulate radio-iodine as a function of the amount of astatine previously administered was determined in two ways. First, the radio-iodine content was measured by detection of the gamma rays by an end-window Geiger counter after the specimen had been fixed in 80 per cent alcohol. The technique was such as to preserve the tissues for subsequent histologic and radioautographic preparations. Second, after the radio-iodine assay, serial sections of each thyroid gland and adjacent tissues, 5 microns in thickness, were obtained by the usual paraffin impregnation technique. Contact radioautographs were made, and a number of sections from the 70-microcurie group were mounted on NTB stripping-film emulsion. The radioautographic and histologic procedures are described in the Appendix.

*Effect on other organs.*—When the rats were sacrificed and the thyroid gland and adjacent tissues removed for study, the following tissues were also taken from the 50-, 100-, and 150-microcurie groups and the controls: bone marrow, lacrimal gland, ovary, pituitary, kidney, stomach,

small intestine, salivary gland, adrenal gland, heart, and liver. The spleen and lymph nodes were obtained only from animals in the 100- and 150-microcurie groups and the control group. These specimens were fixed in Bouin's solution and impregnated with paraffin. Five-micron sections were prepared and stained with hematoxylin and eosin. At least two, and sometimes four, sections were prepared from each specimen.

*Effect on the monkey.*—Varying amounts of astatine were injected into the anterior chamber of the eyes of three young monkeys, and a fourth was held as a control. The reason for this unusual mode of administration is explained in the discussion. The first two monkeys received a total of 200 microcuries, and the third animal received 100 microcuries.

The length of time that the astatine remained in the eye was determined by external counting of the x-rays associated with its decay.

The first monkey was sacrificed ninety-one days, and the second 105 days, after the astatine administration. The second monkey was given 100 microcuries of radio-iodine intraperitoneally, twenty-four hours before sacrifice, because when the neck of the first monkey was opened, after removal of the eyes, no trace of thyroid tissue could be found on gross or microscopic examination. The entire trachea of the second monkey was removed and fixed in 80 per cent alcohol; it was then surveyed with a small end-window Geiger counter, covered with a thin lead shield with a center aperture approximately 2 millimeters in diameter. The small section of tissue that contained radio-iodine was dissected away, embedded in paraffin; by use of standard techniques, serial sections were made. They were covered with NTB stripping film by procedures described in the Appendix.

The third monkey was held for observation, to determine if any functional evidence of injury to the thyroid gland would appear.

## Results

*Effect on the thyroid gland of the rat.*—Figure 3 indicates a depression of the radio-iodine uptake as a function of the amount of astatine previously administered. This curve suggests a small depression of uptake at the 10-microcurie level, perhaps indicative of a minimal degree of radiation injury. At the 50-microcurie level, there was a decided decrease in range, which at 100 microcuries dropped almost to zero. A small but appreciable accumulation by the 150-microcurie group was possibly the result of individual variations in uptake. To establish these

values on a more quantitative basis would require many times the number of animals that it was feasible to use at these high dose levels.

The range of uptake of radio-iodine by the thyroids of the control animals and those receiving less than 10 microcuries of astatine was approximately 2 for each group. Greater variations were observed in the 10-, 50-, and 70-microcurie groups, the largest being the factor of

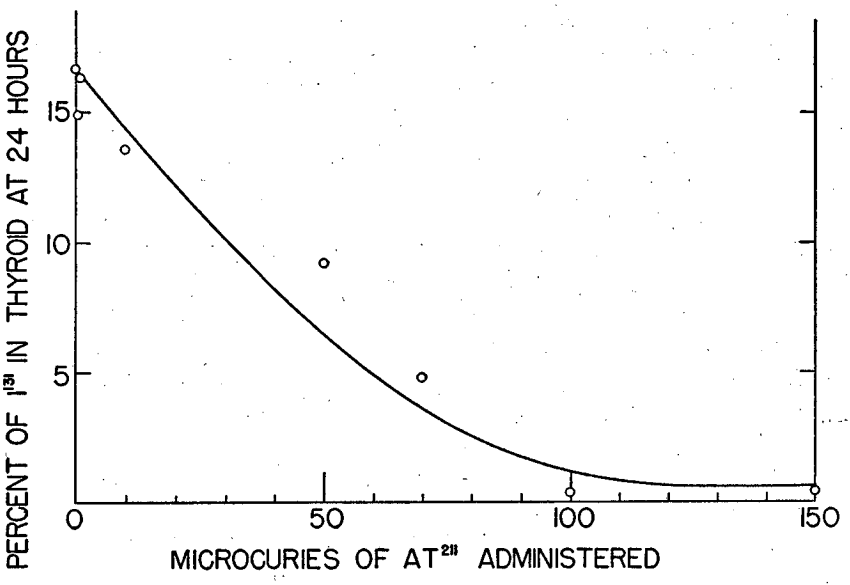


Fig. 3. A correlation between the accumulation of radio-iodine by the thyroid gland of the rat forty-one days after the administration of different amounts of astatine. Seven groups of rats and one control group were given radio-iodine twenty-four hours before the animals were sacrificed. The values shown are the per cent of the administered radio-iodine accumulated by the thyroid gland.

5 in the 50-microcurie group. This effect is interpreted to be due to the observed wide range of astatine uptake by the thyroid and subsequent injury to this organ. The fluctuations noted in the 100- and 150-microcurie groups were less than the factor of 2; this circumstance arose from the fact that at these dose levels there was almost total obliteration of the thyroid.

Four representative tissue sections and their corresponding radio-iodine radioautographs are presented in plates 12, 13, 14, and 15. The radioautograph from the control preparation (pl. 12) suggests quite uniform distribution. The radioautographs from the 10-microcurie

group (not shown) suggested deviation from the normal pattern, insufficient in degree to be clearly seen in a photomicrograph. No observable changes appeared in the radioautographs from rats receiving less than 10 microcuries. It should be pointed out that only the thyroid and parathyroid tissues are shown. The adjacent areas were masked out, since in no instance was there evidence of radiation injury to those regions, and by this means the data are presented more clearly.

Plate 13 shows that radio-iodine deposition in the 50-microcurie group, evidenced by darkening of the emulsion, was quite different from that in the control group. A comparable degree of change was noted in two other animals of this group, but the remaining two did not show so much alteration.

Plates 14 and 15 indicate the deposition of radio-iodine in the 100- and 150-microcurie groups. The marked darkening of certain areas at high dose levels is not necessarily evidence that radiation injury from astatine had not occurred in those regions. The radioautographs indicate only the location of radio-iodine; and the darkening of the film is no quantitative measure, since the duration of the exposures varied. It may be noted that the size of the gland diminished with increasing doses of astatine.

Histologic examination of these sections demonstrated the degree and nature of the injury apparently produced by alpha-particle irradiation. The first indication of possible change was noted in the 10-microcurie group. The epithelial cells seemed to be slightly larger, and there was some desquamation; but in general there was nothing that could be definitely imputed to radiation injury. At the 50-microcurie level, damage was apparent; in general there was a partial destruction of groups of cells without the formation of new acini and some pleomorphism. There was a resemblance to thyroiditis. Similar but more severe changes were seen in the 70-microcurie group. Still more extensive injury appeared at the 100-microcurie level, although occasional acini, usually large, seemed not too severely damaged. In the thyroid glands of the three animals that survived 150 microcuries of astatine there were only seven acini that seemed to have escaped severe injury. Many cells with ovoid nuclei, possibly fibroblasts, were present. The remaining cells tended to be arranged in cords within which a small amount of colloidlike material could be occasionally seen. There were occasional mitotic figures, and a few cells resembled "Hürthle" cells. The parathyroid glands and adjacent tissues in all these groups appeared to be completely normal.

Plates 16, 17, and 18 present a comparison of thyroid and parathyroid tissue from a control animal and from rats receiving 50 and 150 microcuries of astatine. The increase in damage accompanying increase in dosage is shown, but no apparent injury to the parathyroid gland may be seen.

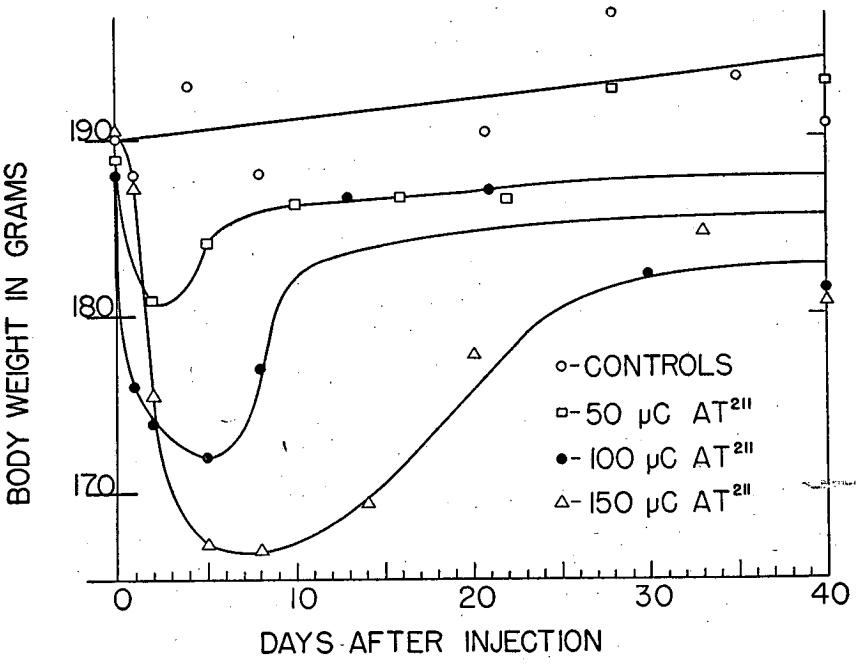


Fig. 4. The changes in body weight of the rats that received by intravenous injection 50, 100, and 150 microcuries of astatine and the control animals.

Plate 19, which is of particular interest, presents a photomicrograph of the radioautograph of a part of the thyroid gland from a rat that had received 70 microcuries of astatine. In the upper right-hand corner are two large acini that apparently not only survived the effects of astatine but had preserved their ability subsequently to accumulate radio-iodine. The dark specks are the reduced granules of silver from the photographic emulsion. Elsewhere in this photograph there is no evidence of radio-iodine accumulation, and there is an extensive degree of damage.

*Radiotoxicity.*—Observations on body weight and the leukocyte count are presented in figures 4, 5, 6, and 7. The decrease in body weight paralleled the fall in the leukocyte count. The subsequent gain in weight

also seemed to be a function of the amount of astatine given, and tended to lag behind the rise in the leukocyte count, especially in the 150-microcurie group.

The change in the leukocyte count was apparent at 50 microcuries and marked at the two higher levels, where the lymphocytes decreased

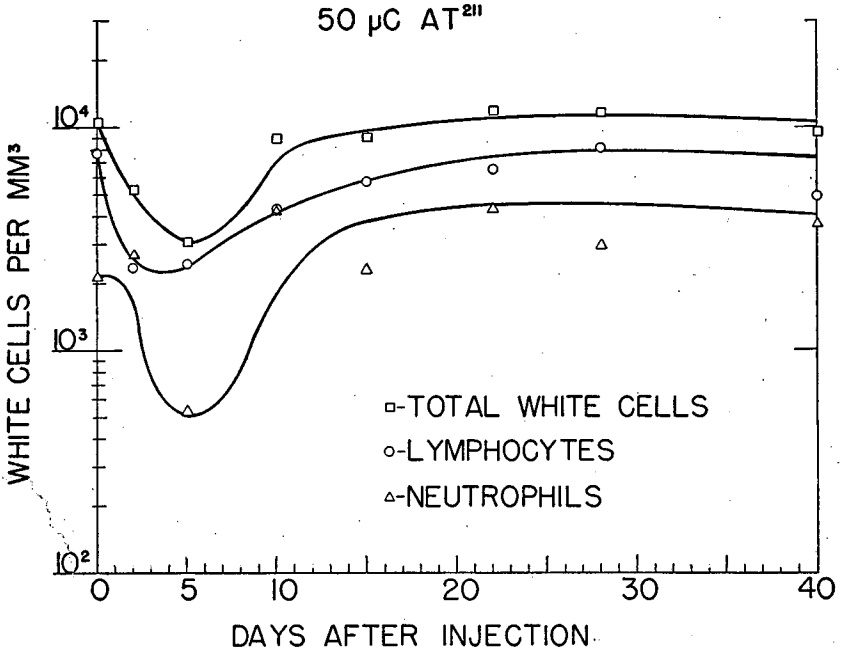


Fig. 5. The depression of the total leukocyte, lymphocyte, and neutrophile counts in the circulating blood of the rat following the administration of 50 microcuries of astatine. Average values for three animals are given.

in number more rapidly than the polymorphonuclear leukocytes. The counts in all three groups again approached the initial values approximately fourteen days after the experiments were started.

A decrease in the erythrocyte count in the 150-microcurie group had only a qualitative significance because of the wide range of individual variation. This group also showed the greatest weight loss; and before recovery began, one rat died. The surviving four showed a marked decrease in food intake; their hair was ruffled, and they were completely docile when handled. The feces were loosely formed, but there was no actual diarrhea and at no time was blood observed. The tails became infected at the site where blood samples were drawn. On the fifteenth

day one of the rats had a profuse bloody discharge from both eyes. At this time its leukocyte count was approximately normal and all four animals had regained to a considerable degree their appearance and normal physical activity. There was no gross evidence of a hemorrhagic diathesis in any of them, and unfortunately platelet counts and other indicative procedures were not feasible. The rat died the following night,

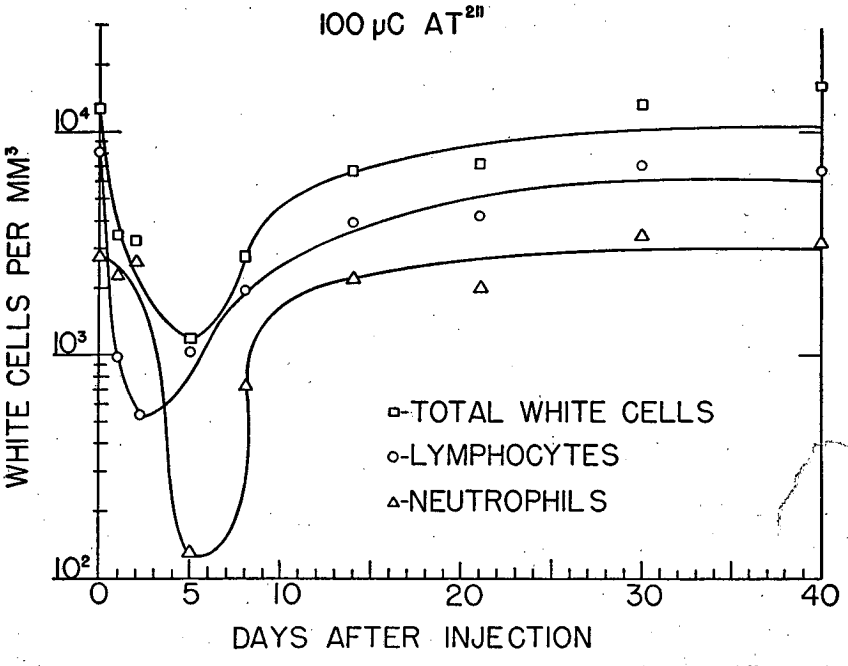


Fig. 6. The depression of the total leukocyte, lymphocyte, and neutrophile counts in the circulating blood of the rat following the administration of 100 microcuries of astatine. Average values for five animals are given.

but autopsy failed to reveal the cause of death. No petechial hemorrhages were observed. Forty-one days after administration of the astatine the three survivors seemed nearly normal in general physical appearance, food intake, and leukocyte counts, and the infected tails had healed.

In animals of the 100-microcurie group, the leukocyte count did not seem significantly different from that of the 150-microcurie group. None of them died, and the physical changes were less severe. No significant drop in erythrocyte count was observed, and there was no evidence of infection or of hemorrhagic diathesis. The feces were more nearly normal. Recovery was more rapid than in the 150-microcurie group.

The rats given 50 microcuries of astatine showed only slight decrease in leukocyte count and a small decline in body weight. This group, and those receiving lower doses, seemed to have the same physical appearance, activity, and food intake as the controls.

*Histopathologic effects.*—Lymph nodes: Alterations in the morphology of lymph nodes following astatine administration are indicated in

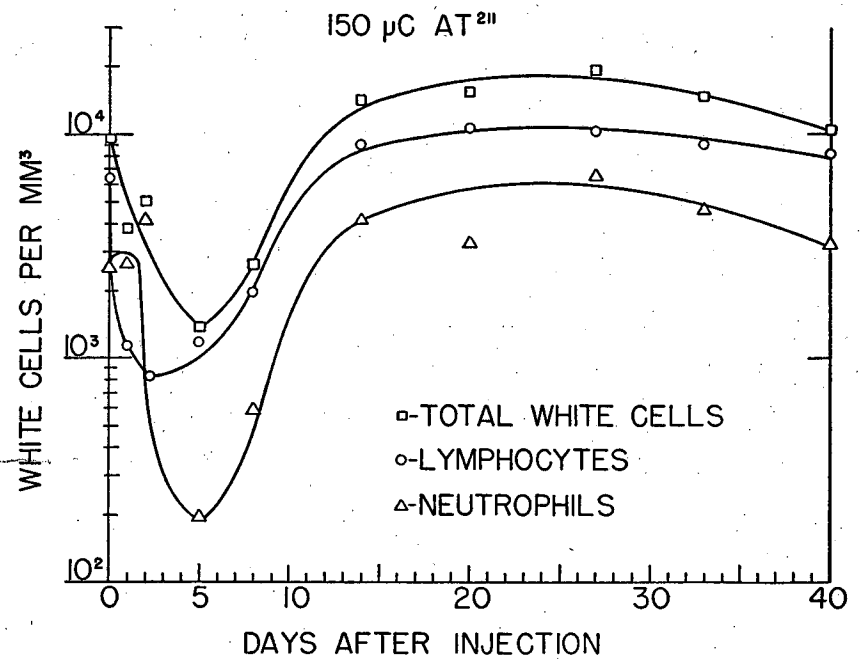


Fig. 7. The depression of the total leukocyte, lymphocyte, and neutrophile counts in the circulating blood of the rat following the administration of 150 microcuries of astatine. Average values for three animals are given.

plate 20. The upper section is from a control animal. In the lower section, from a rat of the 150-microcurie group, the dense layer of lymphocytes about the cortex seems to have been replaced by a layer of fibrotic character. In the medullary region many areas suggested increased lymphopoietic activity. A considerable amount of pigment, very possibly hemosiderin, which was also observed in the specimen, cannot be seen in the photomicrograph. The changes, although present, were much less evident in lymph nodes from rats in the 100-microcurie group.

Spleen: The sections from the 150-microcurie group showed evidence of significant changes. The lymphoid follicles were smaller, and germinal

centers were absent. In some areas large clumps of lymphocytes without germinal centers were noted about the central arteries. There was more pigment than would normally be expected, and Malpighian corpuscles were reduced in size and number. Plate 21 shows a representative comparison of a normal control and an animal from the 150-microcurie group. In the upper section there are two corpuscles quite well defined, with an abundance of lymphoid tissue; whereas in the section below, the normal architecture has been considerably distorted and there seems to be less lymphoid tissue. Changes in the spleen at the 100-microcurie level were minimal and could not be considered definitive.

Bone marrow: Unfortunately there was only one specimen of bone marrow available from the three animals at the 150-microcurie level. This specimen was taken from the vertebral column, and there was no discernible evidence of alteration of cellular activity or changes in the differentiation of the various cell types. The number of megakaryocytes observed was within the normal range. Specimens from the 100- and 50-microcurie groups also showed no discernible deviations from normal.

Lacrimal gland: Abnormalities appeared in the lacrimal glands at all three of the highest dose levels. The deviations were progressive, being most marked in the 150-microcurie group. In general, the acini were considerably smaller and somewhat more numerous; the cells appeared smaller, with less cytoplasm and possibly a greater variation in size of nuclei than in the controls. Areas of edema with infiltration by lymphocytes and polymorphonuclear cells were observed. A few mitotic figures were noted, but there did not seem to be any extensive attempt at regeneration. A noteworthy finding was an increase of dark-staining pigment. In some areas there appeared to be almost complete destruction, although adjacent regions seemed nearly normal. There were also fibrosis and dilatation of the glandular spaces, which were cystic in some areas. In some regions a considerable degree of hyalinization was present. An indication of the degree of injury at the 150-microcurie level is presented in plate 22, together with a section from a control animal. From observations made at the three dose levels, it would seem possible that this effect was due directly to the astatine. The irregularity of the changes, both in different animals and within a single gland, is somewhat puzzling. There were considerable variations in structure, and presumably in functional state, in the normal animals.

Ovary: The ovaries from animals in the 150-microcurie group showed very few developing ova. There were a few distended follicles, lined by very thin, flattened cells, giving the appearance of an extensive

atresia. Some blood was present, both new and old. Sections from the 100-microcurie group seemed very similar, except that occasional large follicles showed more cellular lining and there were some small follicles with apparent atrophic changes. The specimens from the 50-microcurie group resembled the controls.

Pituitary: Sections from the 150-microcurie group showed changes in the anterior lobe but none in the posterior lobe. There was a considerable decrease in the number of eosinophilic cells. A number of the basophilic cells contained relatively few granules, and the cytoplasm was vacuolated. They resembled "thyroidectomy cells," similar to those described by Reese *et al.* (1943) following surgical thyroidectomy. A similar but less pronounced alteration was seen in the 100-microcurie group. The sections from the 50-microcurie group were indistinguishable from those from the control group.

Kidney: The 150-microcurie group presented evidence of slight thickening of the basement membranes of the glomeruli. The cell borders of the proximal convoluted tubules were less clearly defined. Increased pigment was found here, as in many other tissues. Injury to the glomeruli and the convoluted tubules was considered to be questionable. No apparent deviation from normal was noted at the lower dose levels.

Stomach: There was no evidence of inflammatory reaction, and all the various cell types of the gastric mucosa seemed unchanged at all dose levels.

Small intestine: A minimal decrease of lymphoid tissue was noted in the 150-microcurie group. This was not considered to be definitive evidence of change, when compared to either the lower dose levels or the controls.

The salivary gland, adrenal gland, heart, and liver showed no evidence of injury.

*Effect on the monkey.* External counting of the x-rays from astatine subsequent to administration demonstrated rapid absorption from the anterior chamber of the eye. Half the dose disappeared in less than one hour.

When the first monkey was sacrificed at 91 days, no thyroid tissue could be demonstrated, although there had been no signs of thyroid deficiency. The second monkey also showed no evidence of depressed thyroid function when sacrificed at 105 days, but radio-iodine could be located only in one small section of peritracheal tissue, weighing about 200 milligrams. Less than 1 per cent of the radio-iodine administered twenty-four hours earlier remained in the thyroid tissue. Not one follicle

containing colloid was found on microscopic examination, although a few groups of atypical epithelial cells accumulated some radio-iodine. Two representative photomicrographs of the damaged thyroid tissue, together with their stripping-film radioautographs, are shown in plates 23 and 24. It may be noted that parathyroid tissue was present and showed no evidence of damage.

In view of this unexpected effect from a relatively small amount of astatine (approximately 80 microcuries per kilogram), the third monkey, which had received half this amount, was held for observation. Approximately six months after the injection, this monkey began to manifest signs of marked diminution of thyroid function. It became listless, exhibited increased intolerance to cold, decreased food intake, and apparently had less frequent bowel movements than the control monkey. Growth seemed to cease. The monkey was not ill, for when attempts were made to handle it the usual irritability was displayed. As time progressed, pouchiness appeared beneath the eyes and there was a gradual loss of hair. At the end of fifteen months the animal was approximately one-half the size of the control monkey and almost completely hairless (pls. 25 and 26).

To present more convincing evidence that the animal was suffering from hypothyroidism, 6 to 12 milligrams of thyroid substance were given daily in the food. The effect of this regimen was unquestionable. Within three weeks the food intake had almost quadrupled, and the monkey became more aggressive and combative. There was no evidence of toxicity from the thyroid substance, such as tremor or excessive restlessness. The bowel habits returned to normal, as judged by comparison with the control animal. The pouchiness beneath the eyes began to diminish and finally almost vanished. Regrowth of hair began, and at the end of twenty months the pelt seemed almost normal. There was less intolerance to cold, although the monkey still seemed to desire the additional warmth of a small electric heater.

## DISCUSSION

The tracer studies have amply demonstrated the ability of the thyroid gland of the rat and monkey to accumulate astatine selectively. Although the concentration in both species was only about one-tenth that of radio-iodine, the degree of selective localization was still high in comparison to other tissues. Astatine was released somewhat more rapidly from the thyroid gland of the rat than radio-iodine; moreover, the astatine concentration in the thyroid gland has been shown to rise while

the plasma level declined (table 2). The nonuniform distribution of astatine within the different acini is interpreted to indicate that a significant proportion of astatine retained by the thyroid gland was not completely labile. Inasmuch as astatine is the last member of the halogen group, an attractive hypothesis would be that it is organically bound in a manner analogous to iodine. Unfortunately there is no evidence available at present to confirm the validity of such a concept.

The behavior of astatine in the thyroid gland may be explained by the following hypothesis. Astatine may be postulated to enter the epithelial cells from the plasma in the minus 1 valence state. It is reasonable to predict that these cells may be capable of concentrating astatine in this state. The astatine could then be oxidized to the zero valence state in a manner analogous to the oxidation of the iodide ion as postulated by Stanley (1948). At this point, astatine$^0$ could be expected either to be retained by the epithelial cells or to diffuse out into the nonvascular colloid, thus not readily being reabsorbed into the blood stream; at the same time additional astatine was collected from the plasma by the epithelial cells. This would lead to a steady increase of astatine in the thyroid gland, even though the astatine level of the plasma was diminishing. The tracer studies present evidence of this concept. This hypothesis avoids any attempts to explain possible mechanisms of the formation of organic compounds of astatine by the thyroid gland, which, although not yet demonstrated, might be analogous to iodo-derivatives of tyrosine and thyronin.

Another line of evidence pointing toward the possibility that astatine can exist in the plasma in the minus 1 valence state is its concentration and apparent secretion by the stomach.

Interpretation of the results of metabolic studies of radio-iodine, which were done for comparison with the metabolism of astatine, requires consideration of the synthesis and release into the blood of organic compounds of iodine from the thyroid gland, such as thyroxine (Taurog *et al.*, 1950). Since the metabolism of thyroxine seems to be different from that of iodide ion (Le Blond, 1949), tracer data such as have been presented in table 3 might not accurately represent the metabolism of iodide ion because of reëntry of radio-iodine into the blood stream from the thyroid gland incorporated into thyroxin. This phenomenon can be relatively rapid (Chaikoff and Taurog, 1949; Scott *et. al.*, 1951). The fact that the red cell/plasma ratio fell only slightly at the 24-hour time interval suggests that, excepting the thyroid gland, most of the radio-iodine present existed as an iodide ion.

The data shown in table 4 may be interpreted to indicate that there was a rapid equilibrium of an unknown nature between astatine in plasma and astatine in most of the tissues. The radio-iodine data likewise suggest a rapid equilibrium of iodide ion. The reason for this interpretation is the fact that plasma and tissue content diminished with time, but the tissue/plasma ratios remained relatively constant with the exception of the thyroid gland. Table 5 is interpreted to indicate the relative degree of selectivity of different tissues for astatine as compared to radio-iodine. The high values for spleen, adrenal gland, lung, and ovary are surprising in view of their morphological and functional dissimilarities. In many respects, the metabolism of astatine is quite unlike that of radio-iodine. The only apparent resemblance was seen in the thyroid accumulation, gastric secretion, and excretion by the kidneys; even here there were significant discrepancies. The hypothetical equilibrium reactions presented schematically in the Appendix may conceivably offer some explanation of its unique metabolic characteristics. The present knowledge concerning the biochemical reactions of astatine is too meager to warrant further speculation.

There was a correlation between the apparent radiation injury and the functional capacity of the thyroid gland to accumulate radio-iodine. The data do not indicate whether the damaged tissue was capable of producing either the thyroid hormone or one of its iodinated precursors; the radioautographs merely indicated areas where radio-iodine accumulated and was retained. When the amount of astatine administered to rats was 50 microcuries or more, there was a tendency for the radio-iodine to accumulate in the larger acini about the periphery of the thyroid gland. This is believed to be the result of the failure of these large acini to have accumulated a sufficient amount of astatine to cause their destruction. Thus these surviving acini were able to accumulate and retain subsequently administered radio-iodine. The presence of cells in the severely damaged thyroid tissue resembling "Hürthle" cells may have conceivably arisen from excessive stimulation of the few remaining epithelial cells by the thyrotropic hormone.

A preliminary report by Hamilton *et al.* (1950) stated that considerable damage to the thyroid gland in the rat was observed thirty days after the administration of as little as 10 microcuries of astatine. The later studies described in this report did not observe the same degree of injury at this dose level. The early experiments were not quite comparable, and there should be taken into consideration the wide variations in uptake of astatine by the thyroid gland.

Goldberg *et al.* (1950) studied the histopathological changes induced in the thyroid gland of the rat after the administration of large amounts of radio-iodine. The experiments in which the rats received 875 microcuries of radio-iodine and were sacrificed four weeks later most closely approximated the astatine studies in time interval and degree of thyroid damage. The radio-iodine-treated animals showed some injury to the parathyroid gland and adjacent peritracheal tissues. This circumstance arose from the much greater range of beta particles of radio-iodine as compared to the alpha particles from astatine. The destructive effects of astatine and radio-iodine upon the thyroid gland seem to be qualitatively similar except for sparing of occasional acini when astatine was used.

The destruction seemed more complete in the thyroid gland of the monkey, since no surviving acini were to be found, although a few groups of atypical epithelial cells retained their capacity to accumulate radio-iodine. The use of a larger number of monkeys would be desirable to establish whether destruction is actually greater in this animal. The apparent myxedema was comparable to that produced in the dog by large doses of radio-iodine (Goldberg and Chaikoff, 1952). The response to thyroid substance was confirmatory evidence of hypothyroidism.

A precise quantitative calculation of the radiation received from astatine by the thyroid gland of either the rat or the monkey cannot be made from available data, since astatine is apparently not uniformly distributed within the gland and there are marked variations in individual uptake. Furthermore, the rate of thyroid accumulation of astatine approaches its half-life. Only an approximation is possible: assuming an average uptake and retention of 1 per cent, starting at the time of injection in the rat, and a weight of 20 mg., the amount of radiation received at the 50-, 100-, and 150-microcurie levels was 4,300, 8,600, and 12,900 r.e.p.[4] respectively. This computation was based on the calculation that 1 microcurie of astatine undergoing complete radioactive decay in 1 gram of tissues releases 171 r.e.p. The value of $5.7 \times 10^7$ Mev was assumed to be equal to 1 r.e.p., and 6.8 Mev were taken as the average energy of the alpha particles from astatine$^{211}$. The 80 Kev[5] x-rays associated with its decay were not included, since their contribution to the total energy released was relatively insignificant.

The amount of radiation received at the center of the thyroid gland of the rats given 875 microcuries was estimated by Goldberg *et al.*

[4] Roentgen-equivalent-physical.
[5] Thousand electron volts.

(1950) to be 330,000 r.e.p. The apparent radiation injury observed at the end of four weeks was comparable but possibly greater than that in the rats given 150 microcuries of astatine and sacrificed forty-one days later. Thus it seems that the alpha particles from astatine may be more destructive than the beta particles from radio-iodine, to the rat thyroid gland.

The total body irradiation effects from astatine, evidenced by weight loss and leukopenia, occurred in the rat at dose levels that were lower than anticipated. The nonuniform distribution in body tissues makes impossible a precise computation of the radiation received. Approximate values were computed for the dose levels of 50, 100, and 150 microcuries to be 45, 90, and 135 r.e.p. respectively, assuming uniform distribution and neglecting loss by excretion and thyroid accumulation. The average weight of the rats was approximately 190 grams. The results after administration of 150 microcuries of astatine suggest that this is not far from the median lethal dose, although the number of rats used was too small to have statistical significance. The changes in neutrophile and lymphocyte counts at the 100- and 150-microcurie levels were similar, at corresponding time intervals, to those in rats receiving 600r of 200 Kev x-rays (Stearner *et al.*, 1947). The morphologic appearance of the spleen and lymph nodes thirty-five days after x-radiation was apparently more normal than forty-one days after administration of 150 microcuries of astatine. The qualitative differences between the two types of ionizing radiation, observed under conditions approximating total body irradiation, may be imputed to a greater biological effect of the more densely ionizing radiation from the alpha particles, to selective localization of astatine in regions of the hemopoietic system not apparent from the tracer studies, or to a combination of both.

The astatine disappeared rapidly from the eye of the monkey, presumably entering the blood stream. Half had disappeared in less than an hour. Since the half-life of astatine$^{211}$ is 7.5 hours, the hold-up time in the eye can be considered relatively short, and administration by this route should not give results significantly different from administration by other parenteral routes. The complete disappearance of the thyroid gland of the first monkey led to the radioautographic and limited tracer experiments on two young monkeys described earlier.

The reason for the intraocular injections of astatine in the monkeys was as follows. At the time the rat experiments were being conducted, the Division of Ophthalmology of the University of California School of

Medicine was interested in the possibility of the internal irradiation of retention cysts on the anterior chamber of the eye (Schaeffer, 1952). The eyes of the monkeys were then turned over to Dr. Schaeffer for study.

Upon hypothetical grounds, the most desirable radioactive isotope for internal irradiation of these cysts would be a short-lived radioelement that emitted alpha particles and that did not have any descendants possessing a significant degree of radioactivity. Astatine$^{211}$ seemed to best meet these qualifications.

An adequate interpretation of the biochemical properties of astatine is difficult because of the limited knowledge of the chemistry of this radioelement, which can be prepared only in extraordinarily small amounts and which possesses a very transient existence. There is probably less information of a precise nature concerning the fundamental chemical properties of astatine than of any of the other ninety-seven known elements. This situation is inherent with astatine, and seems to be inescapable. The known and potentially greater multiplicity of its valence states adds to the enigmatic nature of astatine within the complex biochemical environment of a living animal.

## SUMMARY

The experiments reported in this paper have included a study of the metabolism of astatine and its biological effects on rats and monkeys. Special attention was directed to the behavior of astatine in the thyroid gland, owing to its selective localization in that organ in both animals. The distribution of astatine within the thyroid gland was studied by the radioautographic technique, and its concentration was observed to vary in the different acini. The preparation of radioautographs was achieved, and they demonstrated the presence of both astatine and radio-iodine in the same sections of thyroid tissue. The administration of stable iodine in milligram amounts appeared to inhibit the accumulation of astatine by the thyroid gland.

The metabolism of astatine and radio-iodine in rats was compared. The apparent secretion of astatine by the stomach and excretion by the kidneys were somewhat similar to those of radio-iodine. Otherwise, with the exception of the thyroid gland, the metabolism of astatine seemed quite different from that of radio-iodine. Information was obtained from the data which indicated that a rapid eqilibrium existed between the tissues and the plasma for both radio elements.

The biological effects that followed the administration of large amounts of astatine to the rat were investigated in considerable detail.

The subsequent capacity of the thyroid gland to accumulate radio-iodine, its distribution, and, finally, the morphological changes were studied. Complete obliteration of all functional acini was not attained at the highest dose level of astatine, which seemed to be within the lethal range. There was no evidence of injury to the parathyroid gland and adjacent peritracheal tissues. The leukopenia, malaise, and weight loss observed were interpreted to indicate radiation injury. The spleen, lymph nodes, and lacrimal glands showed morphological changes in structure that may have been induced by direct radiation effects from the administered astatine.

Limited studies with the monkey demonstrated the ability of astatine to produce a profound degree of injury to the thyroid gland. A state of apparent simian myxedema was produced in a monkey, and subsequently this effect seemed to have been reversed by the peroral administration of thyroid substance.

Evidence is presented that indicates that the relative biological effects of alpha particles from astatine upon the thyroid gland of the rat may be greater than the published values for the destructive action of the less energetic and more penetrating beta particles from radio-iodine.

## APPENDIX

### The Physics, Preparation, and Chemistry of Astatine

The physical properties of astatine$^{211}$ have been elucidated by Croson, MacKenzie, and Segre (1940); at the end of the interval between 1940 and 1948, however, when work was resumed with astatine for biological studies, the energy of the accelerated alpha particles in the 60-inch cyclotron had been increased from approximately 30 Mev to 40 Mev. Kelley and Segrè (1949) discovered a second radioisotope of astatine that was identified as astatine$^{210}$ with a half-life of 8.3 hours. The nuclear properties of both astatine$^{210}$ and astatine$^{211}$ are summarized in figure 8. In addition to these two isotopes of astatine, there are known to exist eleven more, with half-lives ranging from less than a millisecond to 7.0 hours (National Bureau of Standards, 1950). With the exception of astatine$^{211}$, which has a half-life of 7.5 hours, none of the isotopes of this radioelement possess nuclear properties that make them desirable for use either as tracers or for studies of radiation effects. There are no known stable isotopes of astatine.

When the full energy of the alpha particle beam of the 60-inch cyclotron is used, approximately equal quantities of astatine$^{210}$ and astatine$^{211}$ are produced. The point of concern for biological and proposed human studies lies in the fact that astatine$^{210}$ decays by orbital electron capture to produce polonium$^{210}$, as will be noted in figure 8. This isotope of polonium decays by alpha-particle emission with a half-life of 140 days. Its presence in preparations of astatine for biological and possible human use presents two serious obstacles. The assay of astatine$^{211}$ in tissues can become seriously complicated by the presence of polonium$^{210}$ alpha particles. The second objection to the presence of astatine$^{210}$ is the fact that appreciable amounts of the very radiotoxic polonium$^{210}$ are produced. Polonium$^{210}$ has been shown by Fink (1950) to be more destructive in terms of relative biological effectiveness than radium or plutonium. Thus its presence would render the use of astatine in man unjustifiable.

It should be noted in figure 8 that 60 per cent of astatine$^{211}$ decays to form polonium$^{211}$, which has a half-life of approximately one-half of a second and decays by the emission of alpha particles to form stable lead$^{207}$. The half-life of polonium$^{211}$ is so short that it is presumed to be of no significance in any biological experiments. The remaining 40 per cent of astatine$^{211}$ decays to form bismuth$^{207}$. This radioisotope has an estimated half-life of 50 years, as reported by Neumann and Perlmen

(1951). It is not felt that this circumstance would present a significant problem if astatine$^{211}$ is ever employed for human tracer studies. Microcurie amounts of astatine$^{211}$ can produce only a few hundred thousandths of a microcurie of bismuth$^{207}$, and carrier-free radio-bismuth is rapidly excreted (Scott and Crowley, 1950).

To avoid the production of detectable quantities of astatine$^{210}$, a specially designed target was fabricated that contained a sufficient number of aluminum foils to degrade the energy of the alpha particles from the cyclotron to 29 Mev.

The discovery of astatine involved the use of volatilization technics to separate it from the bismuth target. In practice, it is exceedingly simple to separate astatine from bismuth if the separation does not need to be efficient. The production and isolation of astatine from bismuth on a semiquantitative basis, and in such a manner that it may also be transferred efficiently to solutions of small volume, are not easy matters; however, such a procedure has been devised and reported by Garrison *et al.* (1951).

The chemical properties of astatine have been summarized by Johnson, Leininger, and Segre (1949). Astatine in the zero valence state exhibits some properties akin to those of iodine, one being a high partition coefficient between water and in solvents such as carbon tetrachloride and benzene. The minus 1 valence state is produced by reduction with agents such as sulphite ion and zinc. When astatine is in the minus 1 valence state, it coprecipitates with silver iodide. Positive valence states are not clearly understood. Ferric ion is capable of oxidizing astatine from the zero valence state to a plus valence state, which, however, does not readily coprecipitate with silver iodate.

The earlier work of Corson *et al.* (1940) demonstrated that astatine, unlike iodine, was quantitatively precipitated by hydrogen sulfide in acid solutions using such carriers as bismuth, mercury, silver, and antimony. The chemical properties of astatine in many ways seem more like those of a metal than of a halogen. The equilibrium states in which astatine may exist in an aqueous solution at PH 7 are presented schematically below.

$$\begin{array}{ccccccccc} & & & & At_2^{\circ} & & & & \\ & & & & \downarrow\uparrow & & & & \\ At^- & \rightleftharpoons & At^{\circ} & \rightleftharpoons & AtOH & \rightleftharpoons & AtO^- & + & H^+ \\ & & & & \downarrow\uparrow & & & & \\ & & & & At^+ & + & OH^- & & \end{array}$$

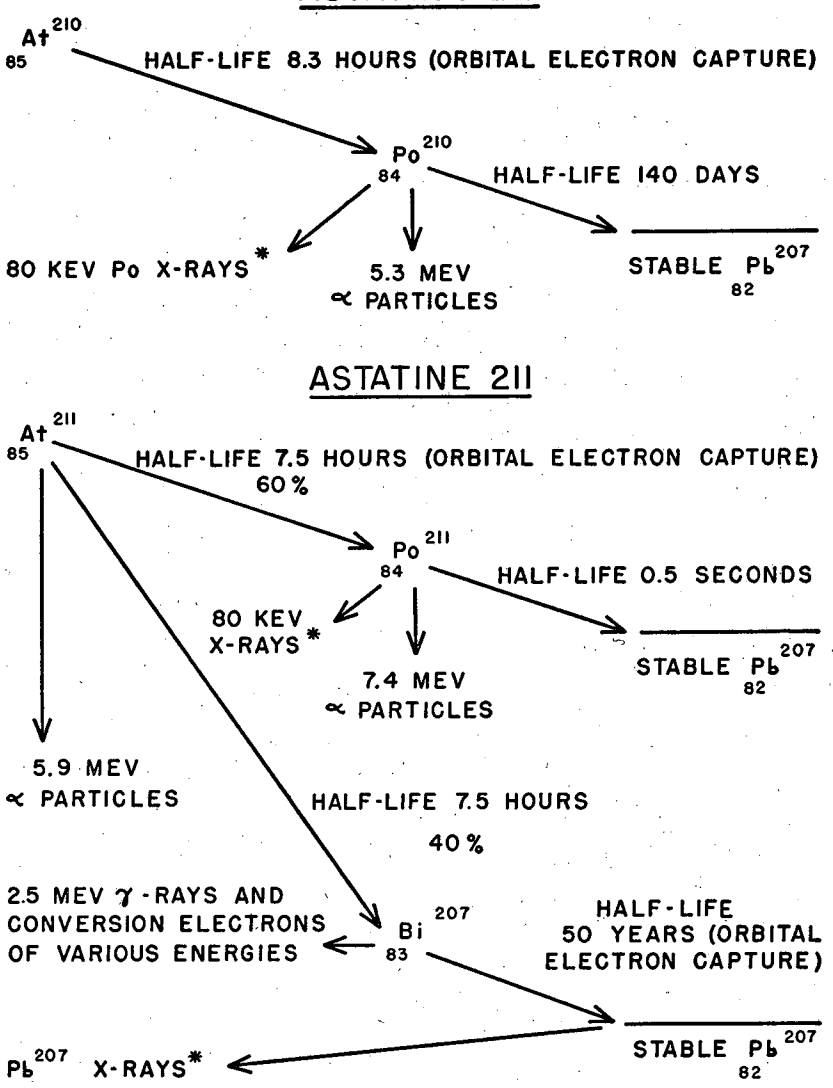


Fig. 8. Decay schemes of astatine[210] and astatine[211].

There is a possibility that all six states may be present simultaneously.

From the practical aspect of working with astatine, the zero valence state was frequently encountered. In aqueous solutions at room temperature at a pH range from 4 to 9, astatine$^0$ has a pronounced tendency to be adsorbed upon most metals. This point was of no small significance, inasmuch as the astatine was sometimes deposited upon the metallic surfaces of the hypodermic needles used to inject this material into the experimental animals. This behavior was erratic and created a troublesome situation. Another undesirable property of astatine at the zero valence state in aqueous solution was its tendency to volatilize at room temperature. Here again the behavior of this substance was somewhat unpredictable. The use of sodium sulfite reduced the astatine to the minus 1 state, which is quite stable and which does not possess the undesirable properties of the zero valence state. When chemical treatment of biological specimens was required for assay of their astatine content, the addition of sodium sulfite was obligatory to avoid losses by volatilization.

Considerable care was taken to establish the radiochemical purity of astatine$^{211}$. After every preparation, a part was set aside for determination of the alpha-particle half-life, as well as the x-ray half-life. In many instances the half-lives for both types of radiation were followed for intervals up to, and occasionally exceeding, 75 hours. In every instance there was no evidence for there being present more than 1 part in $10^6$ of astatine$^{210}$ in the initial preparation.

The reason for measuring the x-ray half-life was that chemically bismuth and antimony are similar and commercially available bismuth may contain traces of antimony. The alpha-particle bombardment of antimony produces iodine$^{123}$, which decays by orbital electron capture and the emission of gamma rays, with a half-life of 13 hours. In those studies that employed the x-rays associated with the decay of astatine$^{211}$ for the assay of biological materials, the presence of iodine$^{123}$ would obviously provide a source of error. Thus, following the half-life of astatine x-rays for extended periods of time ruled out the presence of significant amounts of 13-hour iodine$^{123}$. Likewise, the presence of appreciable amounts of the 4-day iodine$^{124}$ and 13-day iodine$^{126}$ were not observed.

The yield of astatine$^{211}$ was approximately 25 microcuries per microampere hour of 29 Mev alpha particles. This value is for amounts that can be prepared in a form suitable for administration.

### The Methods of Assay of Astatine and Radio-iodine

The measurement of the alpha particles and x-rays associated with the decay of astatine was done by the use of four different types of instruments. The alpha particles were counted by the use of either an argon gas counter or a zinc sulfide scintillation counter. A sodium iodide crystal scintillation counter was developed by Jenkins and Alley (1952) specifically for the purpose of accurate measurement of the 80 Kev x-rays. This device made it possible quantitatively to assay large numbers of biological specimens weighing as much as 10 grams without resorting to any previous chemical processing of the samples. A precedure for the isolation of astatine from such materials has been devised by Garrison *et al.* (1951); it is too laborious and time-consuming, however, when many samples are to be assayed, because of the short half-life of astatine.

In a number of experiments, radio-iodine was employed and both the Geiger counter and the crystal scintillation counter were used. When radio-iodine assays were done, the biological material was not subjected to any chemical processing and the samples were covered by a lead shield of sufficient thickness so that only the gamma rays from the radio-iodine could be detected. In some instances both astatine and radio-iodine were present in the same thyroid tissue. Under these circumstances the astatine alpha particles were measured in the argon gas counter and the radio-iodine gamma rays were determined by use of the Geiger counter. The detection of radio-iodine gamma rays by the Geiger counter was not influenced by the presence of either alpha particles or x-rays associated with the decay of astatine. The samples were covered with a lead shield to absorb the beta rays from the radio-iodine and the x-rays from the astatine. The alpha particles could be determined quantitatively by the argon gas counter, which does not respond either to beta or gamma radiation from radio-iodine or to x-rays from astatine. A direct comparison of the accumulation of the two radioelements was thus possible.

The recent developments and applications of these four different types of counters have been reviewed by Glasstone (1950). Pertinent information concerning the crystal scintillation counter has been presented by Jordan and Bell (1949), Hofstadter (1949), Radio Corporation of America (1949), and Jenkins and Alley (1952). The construction, use, and standardization of the Geiger counter and argon gas counter have been reviewed by Siri (1949).

Frequently the astatine alpha particles were directly assayed in

samples of thyroid tissue weighing less than 100 milligrams. To avoid self-absorption of the alpha particles in the tissue, chemical digestion and evaporation were necessary. Each specimen was macerated in an all-glass homogenizer in the presence of 2 cubic centimeters of 0.1 N NaOH, 1 cubic centimer of 0.1 N NaI, and 1 cubic centimeter of 0.1 $Na_2SO_3$. The presence of sodium sulfite was to insure that the astatine was converted to the minus 1 valence state. The mixture was then transferred to a circular porcelain ashing crucible 4 centimeters in diameter; 1 cubic centimeter of 0.1 N $AgNO_3$ was added, and the material was then heated to 70° Centigrade until all the water had been evaporated. The addition of sodium iodide and silver nitrate served to coprecipitate the astatine with the silver iodide. The preparation was uniformly distributed within the dish to avoid serious errors from self-absorption.

The possibility was considered that this technique might give erroneous results. The astatine content of twenty rat thyroid glands was measured individually, counting the x-rays with the crystal scintillation counter before the fresh tissue was subjected to any chemical treatment. After these determinations the individual samples were digested and dried and the alpha particles counted. The maximum error between the two types of assay for any one sample did not exceed 25 per cent; it averaged 10 per cent. The crystal counter gave values that averaged 10 per cent greater than the alpha-particle determinations. This effect was presumed to have arisen from a small degree of self-absorption of alpha particles in the preparations treated by the chemical procedures described.

### Radioautographic Techniques

NTA and NTB stripping films[6] were used for the preparation of some of the radioautographs by using a modification of the techniques described by Boyd and Williams (1948). NTA stripping film was frequently employed to obtain astatine radioautographs of sections of thyroid tissue from both rats and monkeys. All the rat thyroid glands were fixed in 80 per cent alcohol, dehydrated, cleared, and embedded in paraffin. Five-micron sections were then taken to the darkroom and mounted on the emulsion, using a Wratten Series OA safelight. The film was set on edge in a grooved block inside a light-tight box to allow excess water to drain off. Because the the amount of astatine present in such thin sections was small, the exposure interval was usually twenty-four hours, at the end of which time only a small fraction of the astatine

[6] Nuclear Track Alpha and Nuclear Track Beta stripping film, (Eastman Kodak Company).

originally present still remained. The films and adherent sections were removed from the box in the darkroom, using the safelight mentioned above. They were next immersed in xylol to remove paraffin from the section; successive washings followed, using alcohol that contained increasing concentrations of water, until they were finally placed in distilled water without alcohol. The purpose of this procedure was to hydrate the tissue sections to enable the developer to penetrate to the emulsion and develop the underlying radioautograph. The film was developed in Kodak D-19 full-strength developer for five minutes at 68° F., washed, and cleared with sodium thiosulfate. At the conclusion of this procedure the film was washed in running water for thirty minutes.

The sections on the film were stained with Harris hematoxylin with a modification of the addition of glacial acetic acid in sufficient quantity so that the stain contained 10 per cent of acetic acid. The purpose of this step was to prevent the emulsion from becoming too blue and yet to produce a clear stain of the cell nuclei. The specimens were then placed in 0.25 per cent eosin and 70 per cent alcohol for five minutes and subsequently in 80 per cent alcohol to remove as much eosin as possible from the emulsion without removing too much from the tissue. This balance is a somewhat delicate one for which no specific rules can be given but which must be acquired by experience. Next, the preparations were dehydrated in 95 per cent alcohol and the emulsion and accompanying section were gently stripped free from the heavy backing support. The stripped emulsion and adherent section were then placed in absolute alcohol and cleared with xylol. The excess emulsion was trimmed from around the sections, which were mounted on standard microscope slides, using the conventional cover slips and clarite to obtain a permanent preparation. The same technique was employed for NTB stripping film except that a Wratten Series 2 safelight was used. This substitution was obligatory since this emulsion is very sensitive to light. NTA emulsion is to be preferred for the visualization of alpha-particle tracks, but it will respond to beta particles. When a higher degree of sensitivity to beta particles is required, the NTB emulsion should be used.

An attempt was made to prepare a dual astatine radio-iodine radioautograph from thyroid tissue of a monkey that had received both radioelements. The tissue was fixed in Bouin's solution for thirty minutes, dehydrated, cleared, and embedded in paraffin, and 5-micron sections were cut. The sections were then mounted on NTA stripping film and subjected to the same procedure as previously described for the

preparation of astatine radioautographs of the rat thyroid. The only variation from this method was that individual preparations were developed at different time intervals to achieve an optimum number of alpha-particle tracks from the astatine without having the tracks too heavily overshadowed by the silver granules in the emulsion, produced by the radio-iodine beta particles.

Contact radioautographs were used extensively when radio-iodine was present in the thyroid tissue, particularly with serial sections, because of their ease of preparation. These sections were mounted on large glass slides, and the paraffin was removed by washing in xylene. After the xylene had evaporated, the slides were dipped in a dilute solution of celloidin and set on edge to dry. This procedure served a dual purpose in protecting the sections and also preventing paraffin from coating the emulsion of the No-Screen x-ray film against which the sections were firmly mounted. If the images were too faint, the specimens were exposed for a longer interval of time. Conversely, if the film seemed overexposed, a shorter time interval was employed. It was frequently possible to secure as many as four sets of radioautographic images from a single specimen, which at times made the correlation of the deposition of radio-iodine within the individual follicles much easier. When a maximum number of radioautographs had been obtained, the sections were washed in alcohol to remove the celloidin and stained with hematoxylin and eosin.

The question of leaching had to be considered in the radioautographic preparations with astatine. When 80 per cent alcohol was used as a fixative, approximately one-half of the astatine was leached from the thyroid gland of either the rat or the monkey. Very little additional loss occurred during dehydration and clearing of the preparations. The use of Bouin's solution reduced the leaching; there seemed to be considerable translocation of astatine within the thyroid tissue, however, which tended to smear the preparations with a large number of tracks, thereby producing a background effect that made the interpretation of the radioautographs more difficult. Thus it would seem that 80 per cent alcohol dissolves out the astatine that is relatively labile and leaves undisturbed that which is more firmly bound by the thyroid gland. In view of these observations 80 per cent alcohol is considered to be a more satisfactory fixative than Bouin's solution.

Mr. H. Ralph Haymond and Mr. Donald C. Morrison were responsible for handling many of the difficult problems associated with the isolation of astatine. The physical procedures required for the assay of astatine x-rays in biological specimens were developed by Mr. John C. Alley and Mr. Kenneth Jenkins. A large share of the tracer studies was done by Miss Patricia C. Wallace with the invaluable assistance of Miss Margaret Gee, Miss Marilyn Hemenway, Miss Helen Johnson, and Mrs. Alberta Mozley. The radioautographic experiments that appear in both the tracer studies and the biological effects of astatine were the result of work done by Mrs. Berniece Jue Louie and Miss Gretchen Thilo.

John C. Bowers, M.D., Dean of the University of Utah School of Medicine, Salt Lake City, Utah, and Stuart Lindsay, M.D., Associate Professor of Pathology, University of California School of Medicine, examined most of the histologic preparations, and their suggestions and observations were most helpful. The continued interest and encouragement of Robert S. Stone, M.D., Professor of Radiology, and Hamilton H. Anderson, M.D., Professor of Pharmacology, and Dermot B. Taylor, M.B., Associate Professor of Pharmacology, are recognized with gratitude. Most of the histologic preparations were made by the technical staff of the Department of Pathology and were of material assistance in this phase of the experiments. Dr. T. M. Putnam, Mr. G. B. Rossi, and the staff of the 60-inch cyclotron were responsible for the production of astatine. Discussions with Dr. Emilio Segré, Professor of Physics, concerning the chemistry of astatine were most profitable. The preparation and editing of the manuscript by Mrs. Marjorie S. García and Dr. Mary K. Dunlap and the stenographic assistance of Mrs. Barbara Butler were invaluable.

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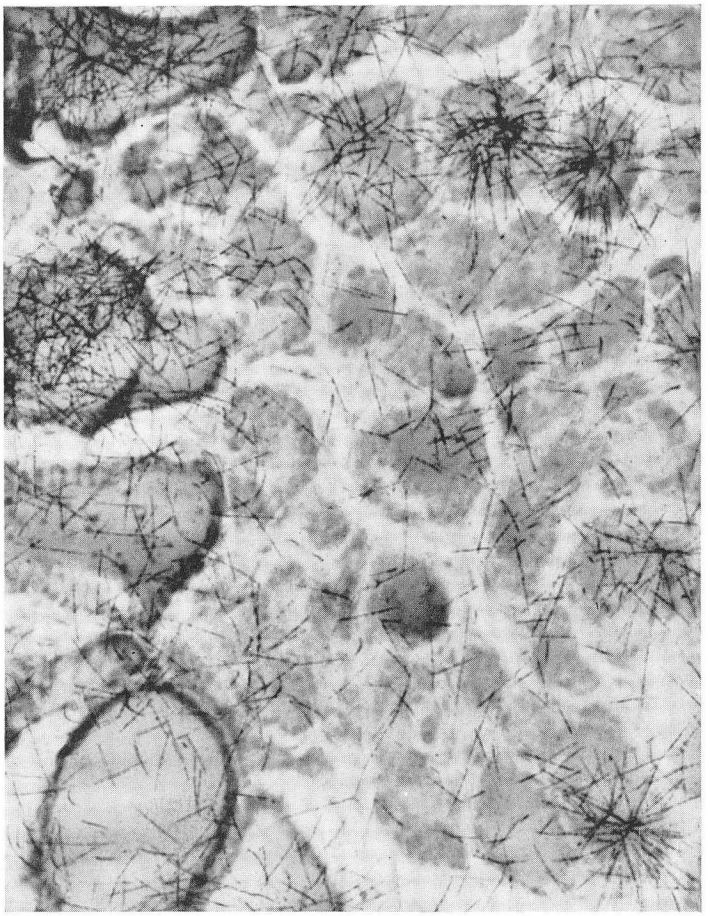
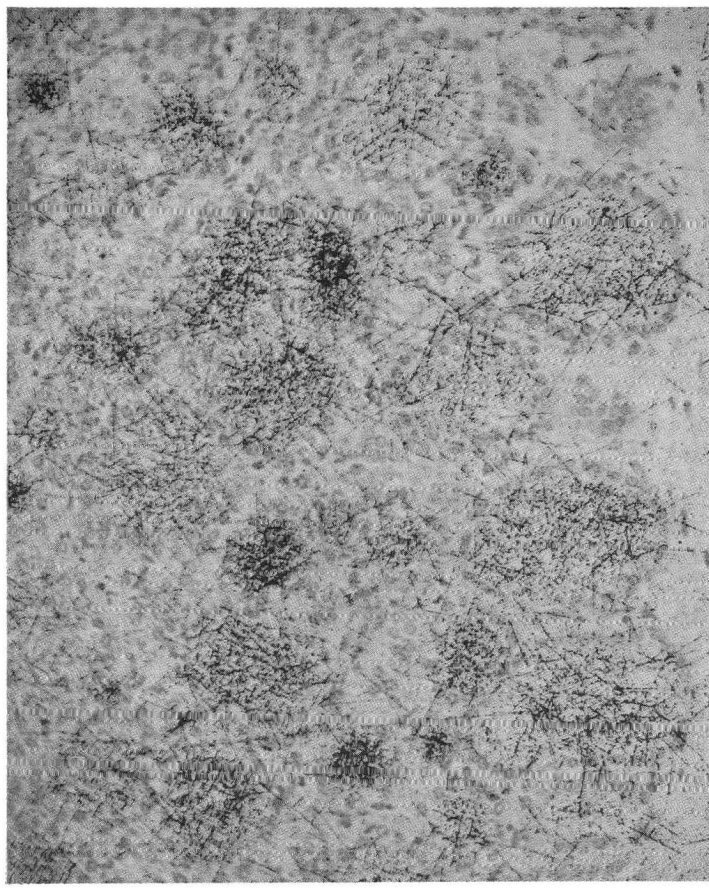
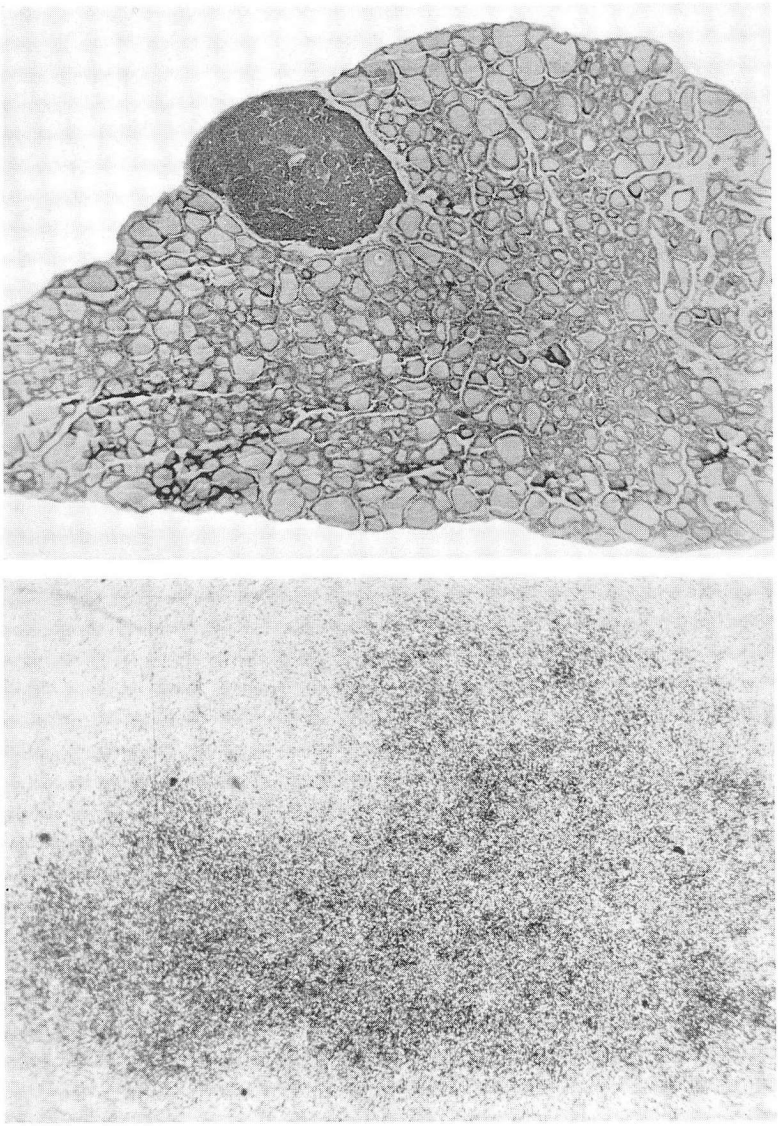
# PLATES



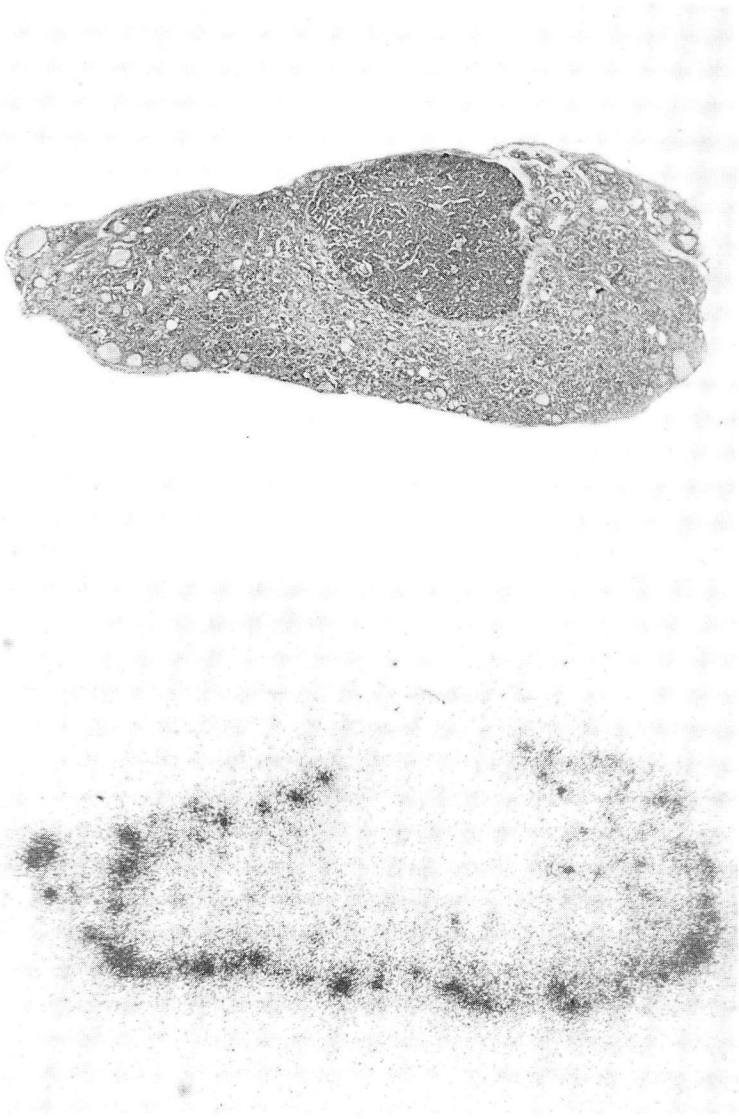
An astatine radioautograph of the thyroid gland of the rat. The presence of astatine is shown by the heavy dark lines which were produced by the alpha-particles which arose from its decay. The irregular deposition in different acini is quite evident, (×300).



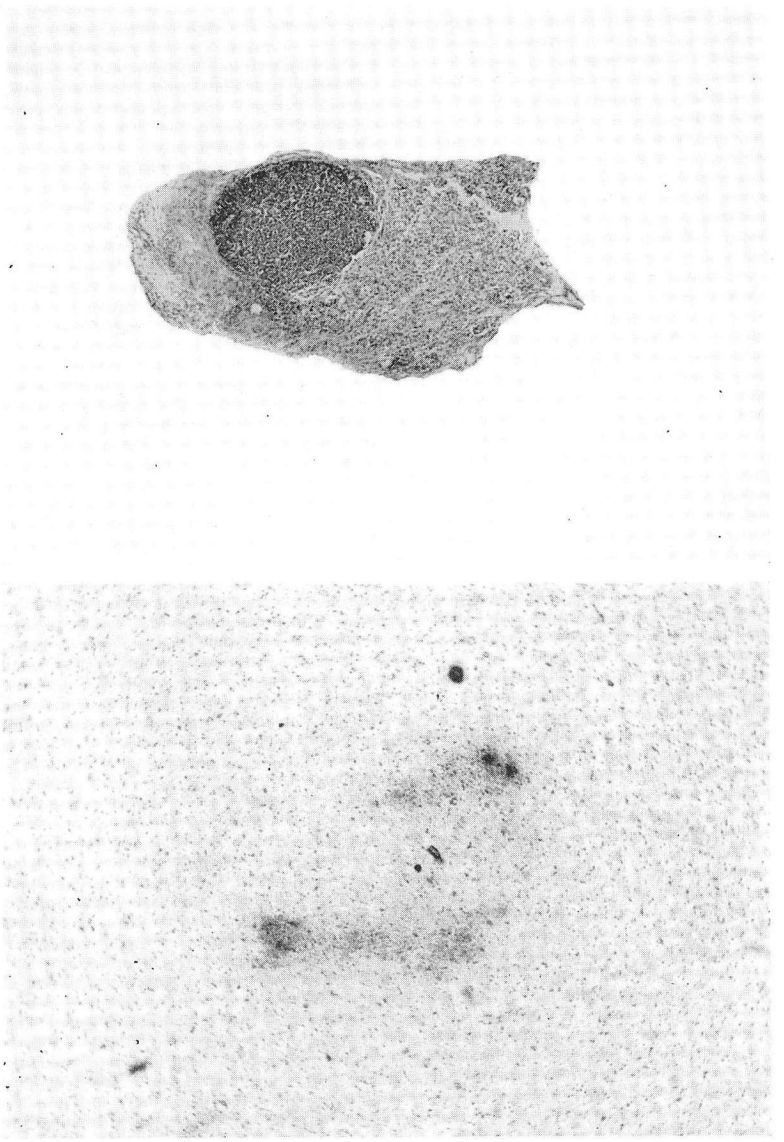
Dual radioautograph of astatine and radio-iodine in a part of the thyroid gland of the monkey. The presence of astatine is demonstrated by the tracks of its alpha-particles. The beta rays from radio-iodine produced the fine black granules of reduced silver in the adherent photographic emulsion, (× 300).



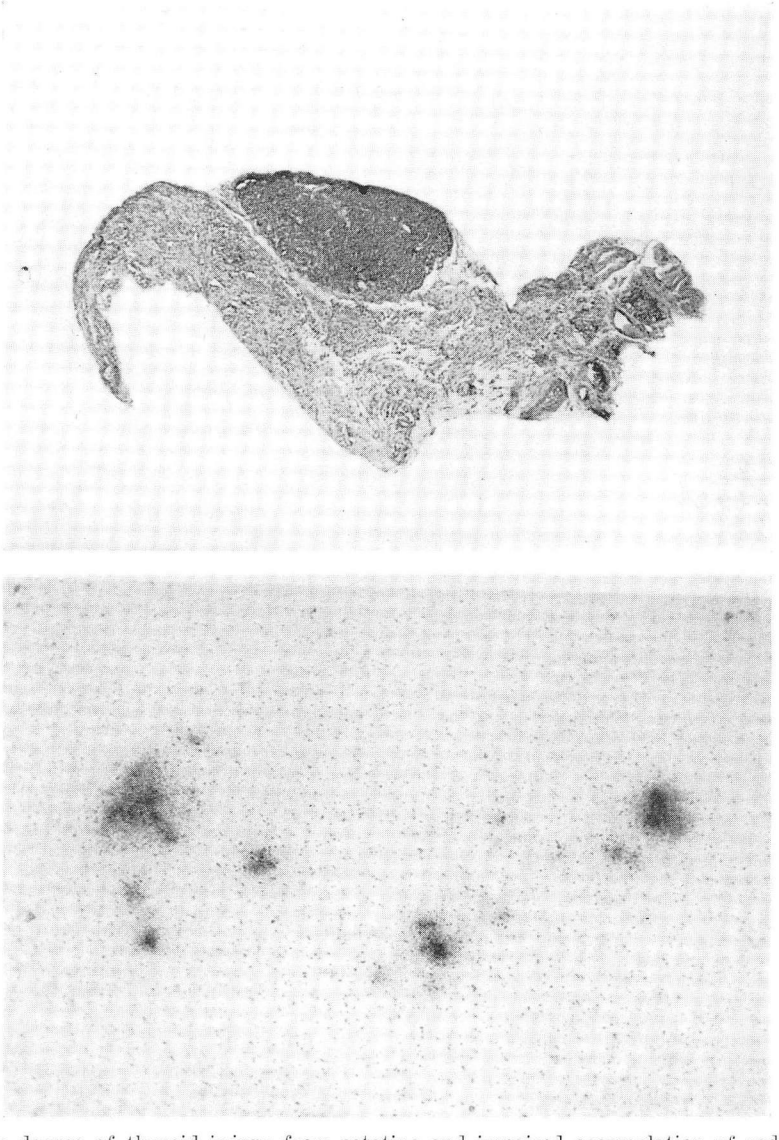
A photomicrograph and corresponding radio-iodine radio-autograph of normal thyroid and parathyroid tissue from the rat. The diffuse distribution of radio-iodine is evident. The apparent lack of accumulation of radio-iodine by the parathyroid gland is to be noted, (×40).



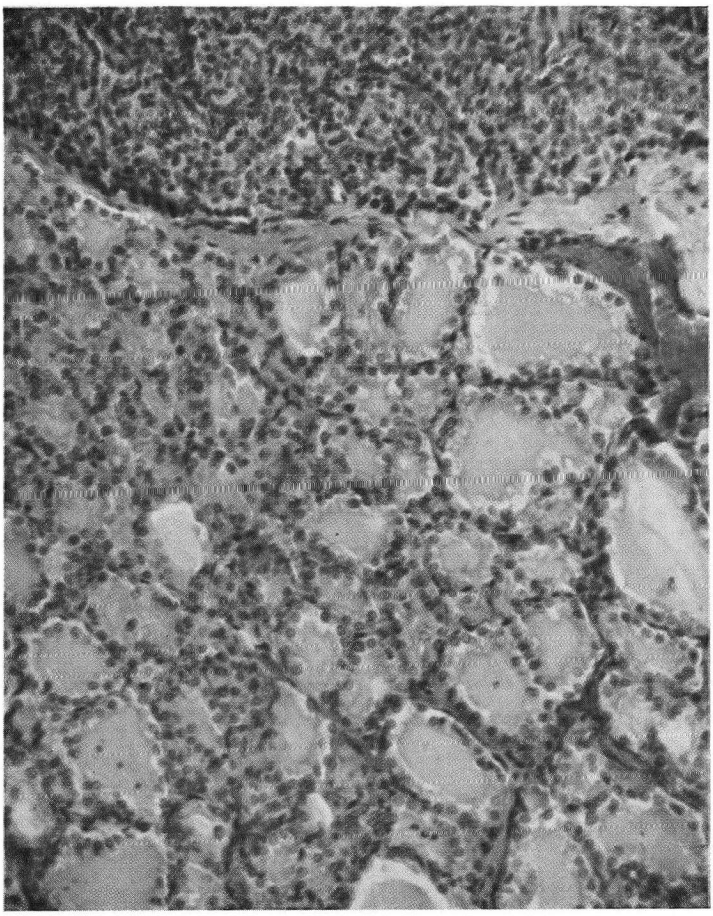
A photomicrograph and corresponding radio-iodine radioautograph of the thyroid gland of a rat which had previously received 50 microcuries of astatine. The destruction of most of the thyroid tissue and decrease in size is apparent. A number of remaining acini are to be seen predominantly about the periphery of the thyroid gland and these acini apparently preserved their capacity to accumulate and retain radio-iodine subsequently administered. The parathyroid gland appeared to be normal, (× 40).



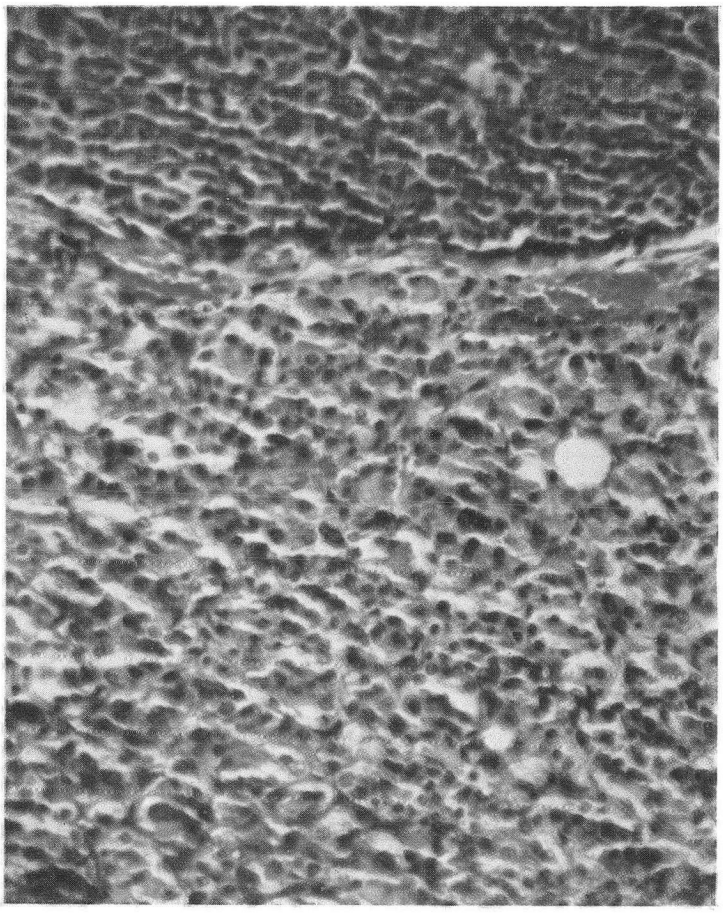
A photomicrograph and corresponding radio-iodine radioautograph of the thyroid gland of a rat which has been given 100 microcuries of astatine 41 days before sacrifice. Only a few acini remain which could accumulate and retain radio-iodine. The general degree of apparent thyroid destruction is more profound. No visible alterations of morphology of the parathyroid gland could be seen, (× 40).



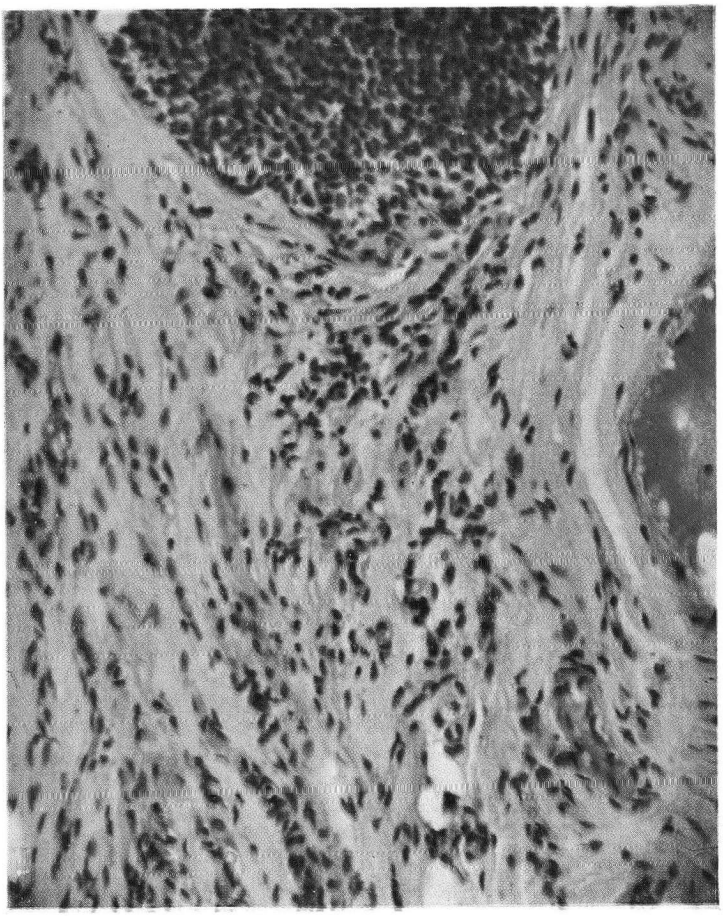
The degree of thyroid injury from astatine and impaired accumulation of radioiodine shown by the radioautograph is indicative of the action of the parenteral administration of 150 microcuries of astatine. Even at this dose of astatine the morphology of the parathyroid gland was apparently unchanged, (× 40).



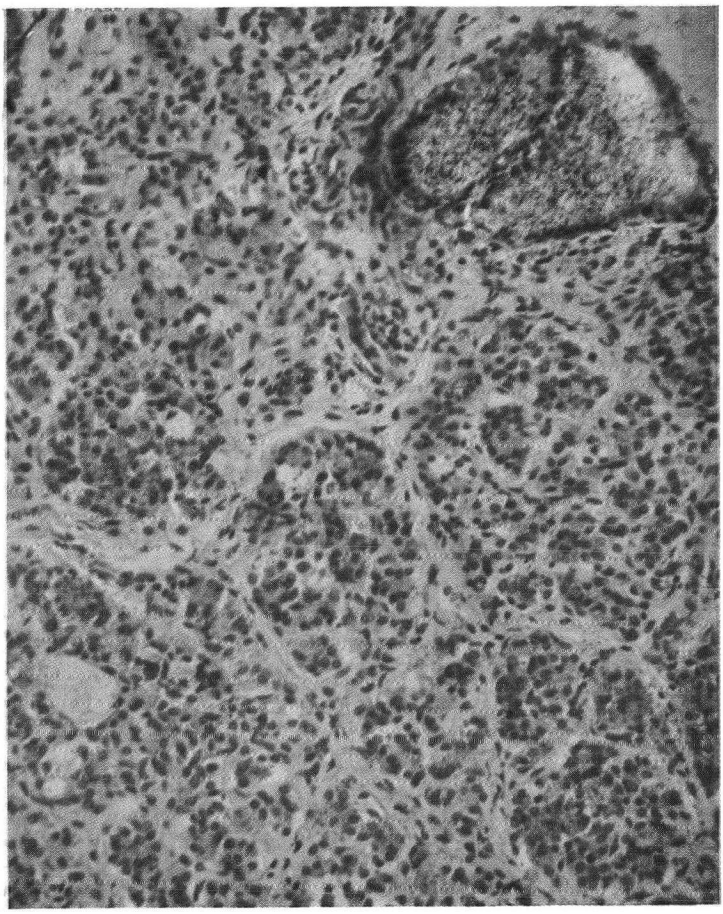
Normal thyroid and parathyroid tissue of the rat, (×300).



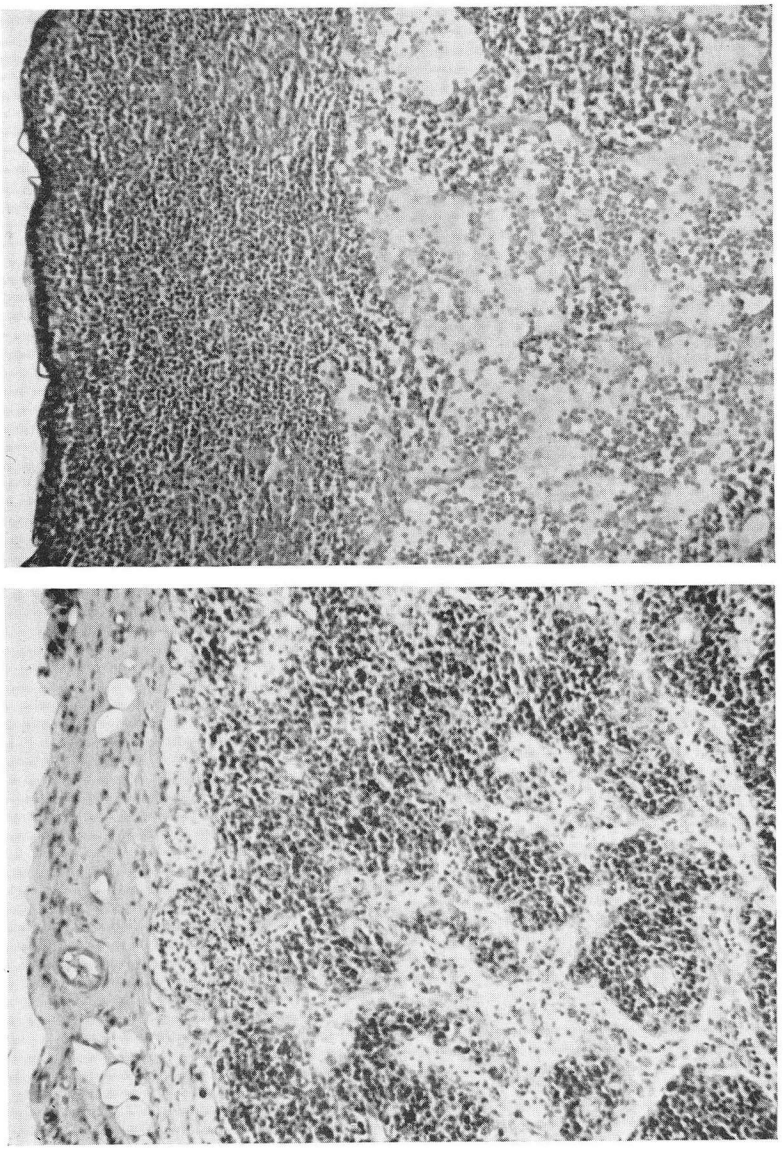
Histopathological changes in the thyroid gland of the rat 41 days following the administration of 50 microcuries of astatine. No follicles are present and no attempts to form follicles can be seen. No changes in the parathyroid gland are apparent, (×300).



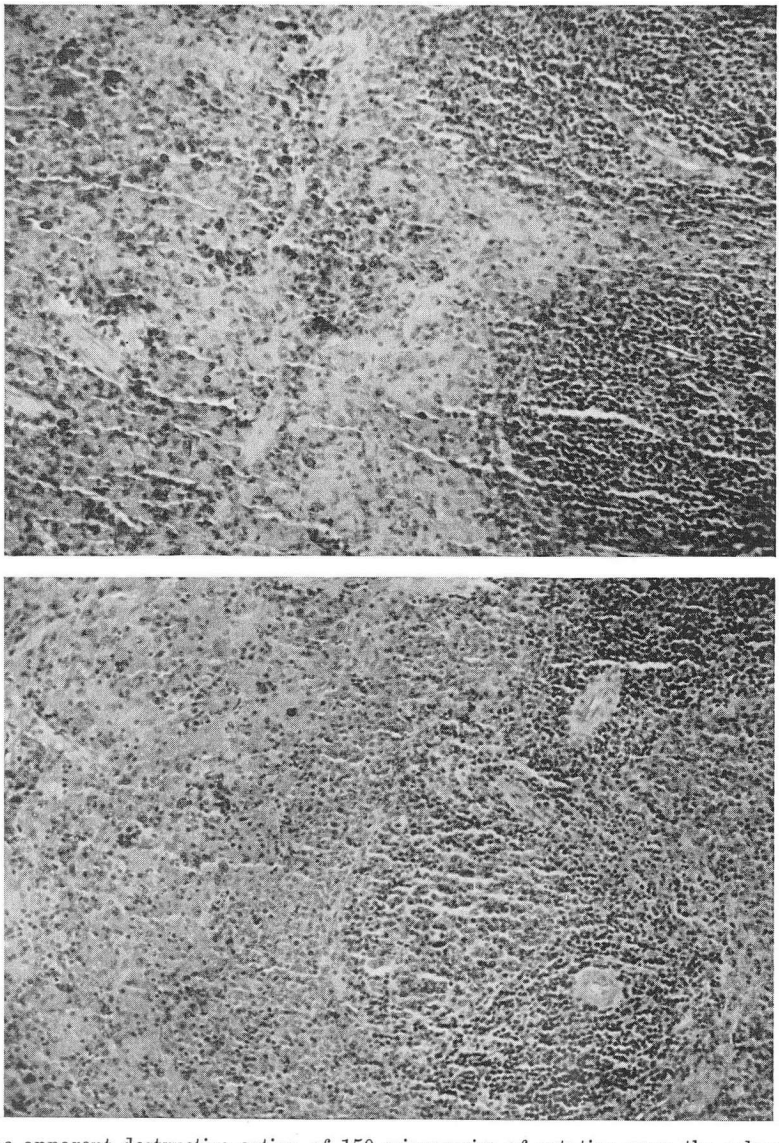
The action of 150 microcuries of astatine upon the thyroid gland of the rat shows the profound degree of destruction that occurred. The eosinophilic structure on the right is a large blood vessel. The parathyroid gland apparently escaped injury, (×300).



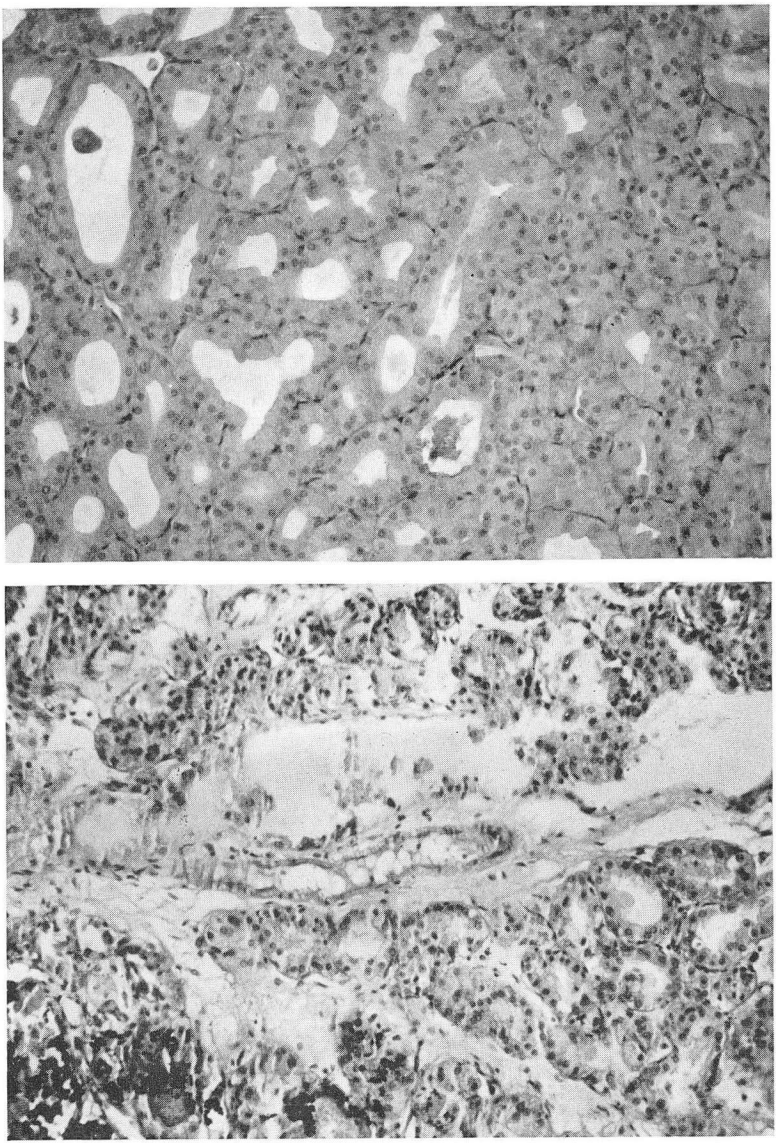
The thyroid injury from 70 microcuries of astatine. Carrier-free radio-iodine was given 24 hours before sacrifice and an NTB stripping film radioautograph was prepared. The two surviving acini may be seen in the upper right hand corner. The accumulated and retained radio-iodine produced the black granules of reduced silver halide within the photographic emulsion beneath these two acini, (×300).



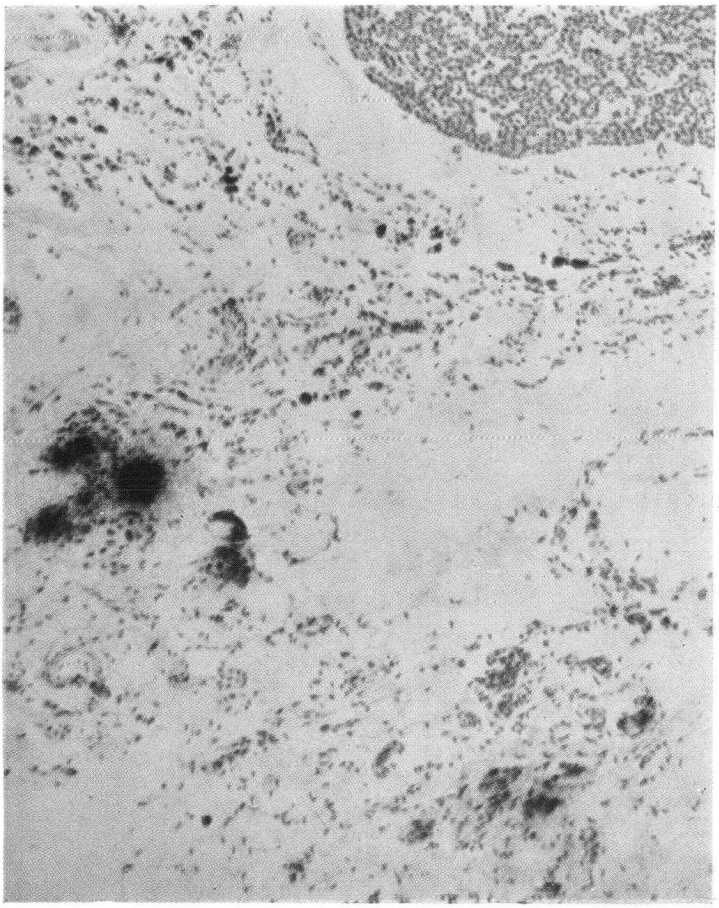
A portion of a cervical lymph node from a rat which had received 150 microcuries of astatine 41 days prior to sacrifice. At the top is a section of a lymph node from a control animal. Degenerative changes and areas of lymphopoietic activity may be noted in the photomicrograph of the section of the lymph node from the treated animal, (×270).



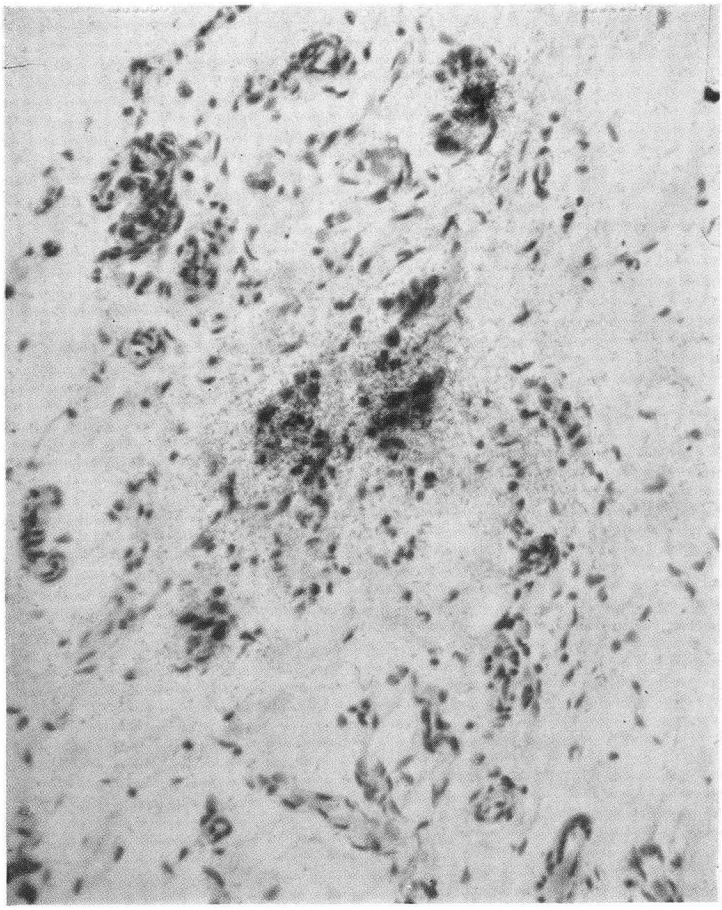
The apparent destructive action of 150 microcuries of astatine upon the spleen is shown in the photomicrograph at the top. The distortion of the normal morphology, apparent diminution of lymphoid tissue, and absence of Malpighian corpuscles in this section may be compared to the photomicrograph below from a control animal, (×270).



A comparison of the normal structure and apparent morphological alterations of the lacrimal gland of the rat which are presumed to have been produced by the administration of 150 microcuries of astatine to the treated animal. The photomicrograph of a specimen from a control animal is at the top, (× 270).

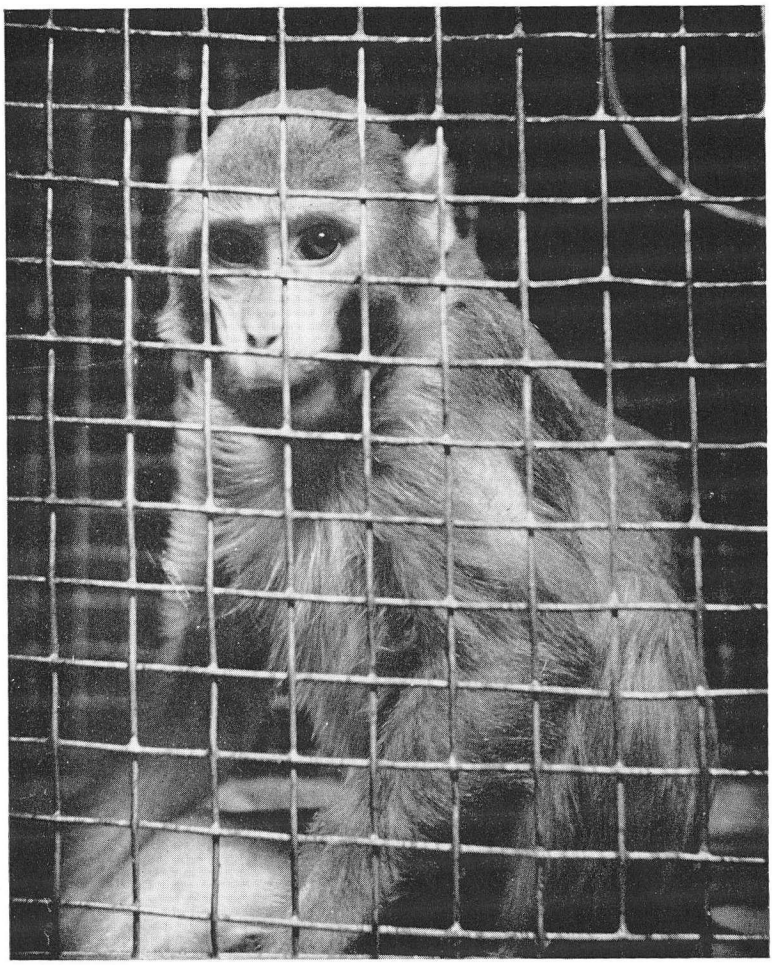


A section and radio-iodine radioautograph of thyroid and parathyroid tissue from a young rhesus monkey which had received 200 microcuries of astatine 105 days before sacrifice, and was given 100 microcuries of radio-iodine the preceding day. The complete obliteration of the thyroid tissue is evident. The dark areas indicate regions of accumulation and retention of radio-iodine. No observable effect upon the parathyroid gland was noted as may be seen at the upper right hand corner, (×120).



A higher magnification from lower right hand area of the section shown in Plate 14. The individual silver granules of the underlying radioautograph can be seen. The cells do not morphologically resemble normal epithelial cells of the thyroid gland although they apparently possessed the ability to accumulate and retain radio-iodine. The absence of pigment, inflammatory changes, and limited fibrosis is noteworthy, (×300).

The appearance of a young rhesus monkey 15 months after receiving 100 microcuries of astatine. The apathetic posture, loss of hair, and pouches beneath the eyes are evident.



The control animal which had not received astatine and was originally the same size, as the injected animal. The differences in size, pelt and facial appearance are evident.