RADIOIODINE: A METHOD FOR MEASURING THYROID ACTIVITY

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THIS paper describes a method for measuring the uptake and retention of iodine 131 in the thyroid glands of living birds. It is hoped that this method may prove useful in determining annual cycles of thyroid activity. A preliminary investigation of the relationship between the level of radioiodine in the blood and in the thyroid glands at various intervals after injection was also begun at this time.

Materials and Methods.—The 16 birds used in the tests—2 White-crowned Sparrows (*Zonotrichia leucophrys pugetensis* and *Z. l. gambeli*) and 14 Brown Towhees (*Pipilo fuscus*)—were trapped as needed in the Santa Barbara area. They were weighed, banded, and put in a large cage prior to being injected with radioiodine. Plain canary seed, young grasses, and water were kept constantly before the birds. To allow the birds to adjust to their new surroundings, they were held a minimum of 4 days prior to being injected. The birds in the third series of tests were held almost 16 days, because of an unforseeable delay. Upon completion of a test-series, the birds were aged, weighed, and set free. Determinations of sex could not be made, since males and females look alike in both species.

Iodine 131 was diluted in 0.9 per cent saline solution to a concentration of 5 microcuries (μc) iodine per 0.25 cc. saline. This solution was then injected into the posterior margin of the right *pectoralis* muscle of the bird. All materials and equipment were sterilized before injection.

Radioactivity was determined by means of a P-20A Scintillation Detector tube containing a gamma crystal. The counts were recorded by a "Tracerlab" scaling device. The counts of radioactivity taken at any one time were recorded in such quantity or for such a period of time as to insure a margin of error of less than 1 per cent. Since mechanically the scaling device is unable to record all counts of radioactivity above 15,000 counts per minute (CPM), the number missed being in direct proportion to the increase in radioactivity above this level, readings higher than this may be corrected by the "split source" method. The readings in this paper have not been so corrected, however, as the mere presence of a high peak was sufficient. Extensive quantitative analyses are beyond the scope of this paper.

The Scintillation Detector was housed in a lead, shot-filled casing, and rested on a circular, lead chamber-house which had a hole in the top to accommodate the crystal face of the Detector. Since it was necessary to make the two-inch diameter of the Detector face smaller, so as to be able to localize the area of the thyroid glands under the apparatus, a lead block containing a hole of 0.8 cm. diameter was used directly below the hole in the chamber-house.

A system of two correlated graphs was used to facilitate the location of any given area of the bird under the Scintillation tube. These graphs were aligned to an accuracy of 0.2 sq. cm. The effectiveness of the lead block, the accuracy of the graphs, and the general dependability of the equipment were rechecked by means of a gelatin phantom injected with amounts of iodine 131 comparable to those introduced into the birds tested.

The exact location of the thyroid glands with respect to the "outside topography" of the birds was determined by a sample dissection. This area was put under the hole in the lead block, and thus under the Detector, and the bird was moved slightly back and forth under the apparatus so as to obtain the highest possible reading of radioactivity. It was then assumed that a true reading of the thyroid area was being recorded.

The bird was held under the Scintillation Detector by means of a specially constructed bird-box, fitted with foam rubber and having a plastic lid. Levers in the box enabled the breast region of the bird to be pressed tightly against the plastic cover. Blood samples, taken from a wing vein, were put in a special plastic holder which fitted on top of the lead block.

All readings of radioactivity are recorded and corrected to "no decay," thus compensating for the decrease in radioactivity readings during the tests, due to the half-life disintegration of the iodine. All of the birds lost weight slightly, probably owing to the rigors of capture and confinement.

Tests.—The first series of tests, Series 1 (Figure 1), was started on April 5, 1956. Five μc's of iodine 131—an amount comparable to that used in Oregon Juncos by Bailey (1953)—were injected into each of two mature White-crowned Sparrows of similar weight, at 8:00 A.M. The radioactivity of the thyroid area was tested one-half

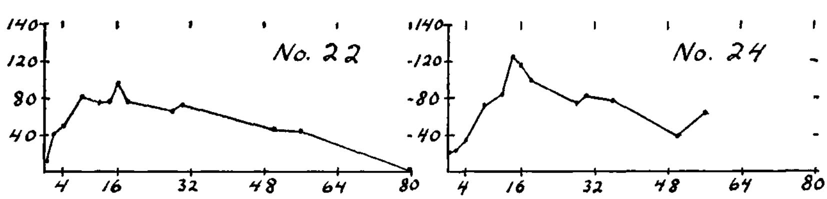

FIGURE 1. Radioactivity of the thyroid areas of 2 White-crowned Sparrows. CPM × 100, ordinate; hours after injection, abscissa.

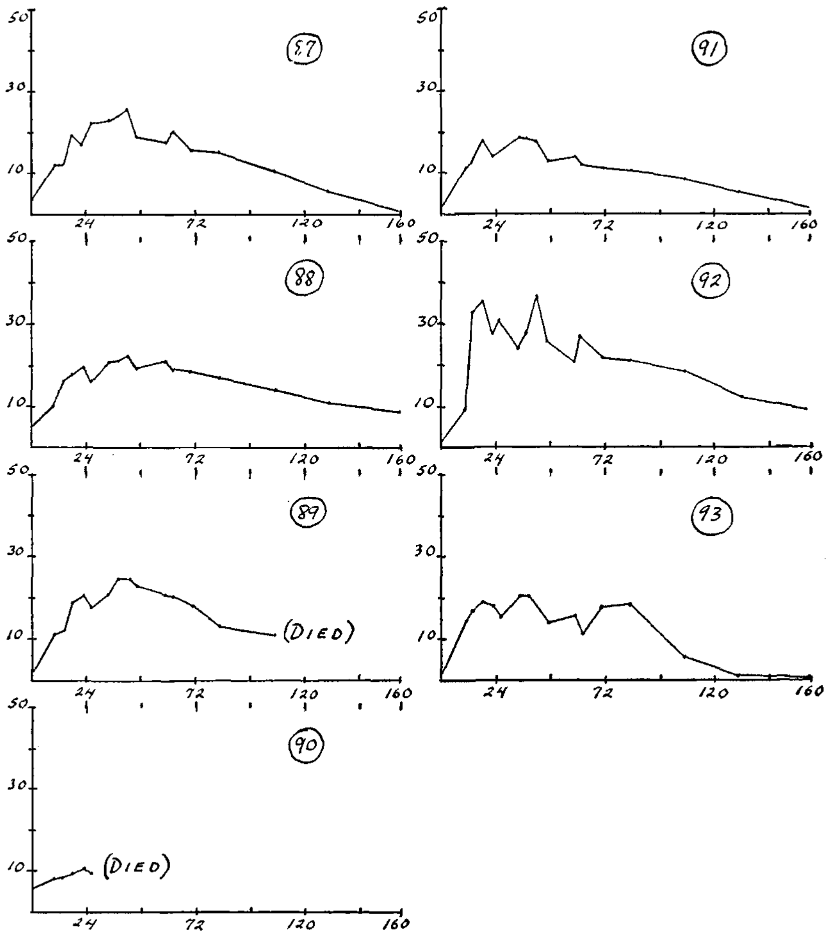

FIGURE 2. Radioactivity of the thyroid areas of 7 Brown Towhees. CPM × 1000, ordinate; hours after injection, abscissa.

hour after injection and at given intervals thereafter. Testing was discontinued at 80 hours after injection when the level of radioactivity was lower than the base rate obtained one-half hour after injection.

Since some preliminary results with blood levels of radioactivity indicated that a dose of 5 μc. was too small an amount with which to obtain readings of sufficient height to be distinguished from the background reading, it was decided to inject an increased amount of radioiodine into the birds of the next series. It was expected that towhees, which were to be used in place of the already migrating White-crowned Sparrows, could probably handle it without harmful effects.

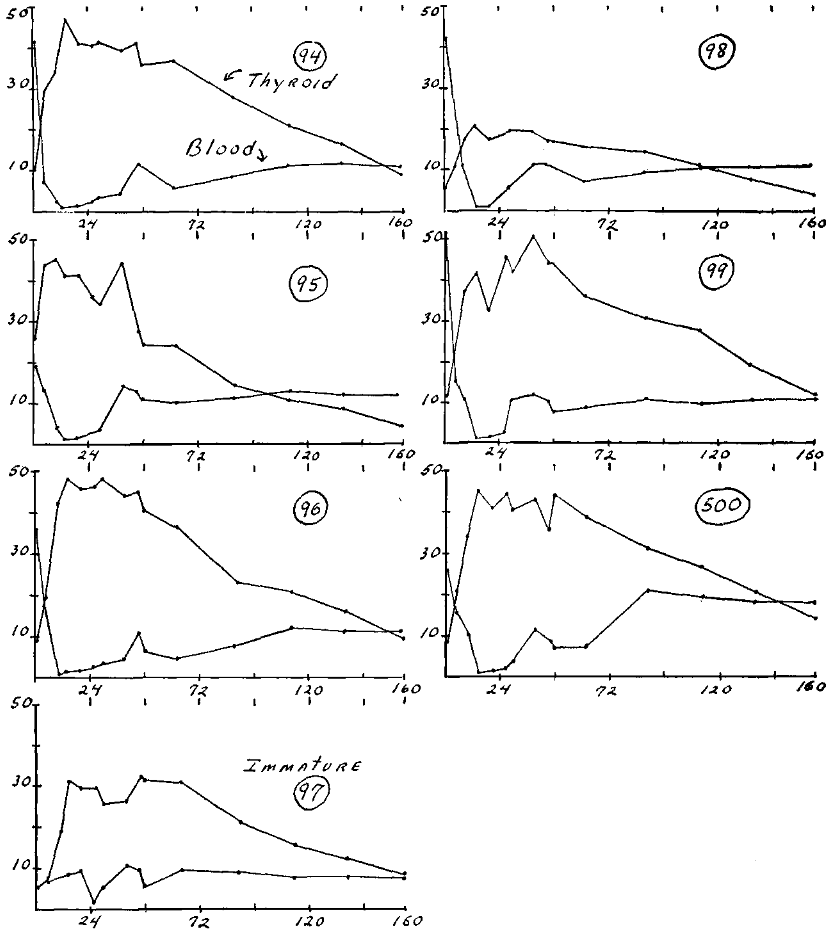

FIGURE 3. Radioactivity of thyroid areas and blood samples of 7 Brown Towhees. CPM × 1000 for the thyroid areas and CPM × 10 for the blood samples, ordinate; hours after injection, abscissa.

Accordingly, on May 4, 1956, Series 2 (Figure 2) was started, using 7 mature Brown Towhees which were injected with 10 μc. each at 9:30 P.M. The birds were aged by observation by Dr. M. M. Erickson. The radioactivity of the thyroid area was tested one-half hour after injection and at given intervals thereafter. Testing was discontinued 160 hours later. There were two casualties. No blood readings were taken.

Series 3 (Figure 3) was begun on May 28, 1956, at 7:00 P.M. A group of Brown Towhees, consisting of 6 mature individuals and one immature, was injected with 10 μc. portions of radioiodine. The birds were aged by examination of the skull as described by Miller (1946). Testing was discontinued 159 hours after injection. Blood readings were taken. There were no casualties.

Results (summarized in Figures 1–3).—Both White-crowned Sparrows reached a peak of radioactivity in the thyroid areas 14 to 16 hours after injection. This activity declined gradually until the end of testing.

In combining the results of the two test-series on the towhees, it was found that 10 birds reached a peak of radioactivity in the thyroid area 17 to 18 hours after injections; 2 birds reached peak activity 22 hours after injection; one, after 13 hours; and one never reached much of a peak and died 34 hours after injection. From the above results, it can be seen that on the whole, the towhees attained a peak of radioactivity in the thyroid area some 2 hours later than did the sparrows.

After the initial rise, fluctuations occurred in the level of radioactivity in the thyroid area until approximately 43 hours after injection. Fluctuations in certain individuals also occurred from 59 to 83 hours after injection. Whether the fluctuations during the first 43 hours were brought about by the close spacing of the test intervals, and whether they might have shown up during the period of gradual decline had that part of the test been conducted also with such intervals, cannot be conclusively determined at this time. The fact that such fluctuations were found in Series 2, where the intervals between tests from 59 to 83 hours were shorter than in Series 3, would seem to support this idea.

The blood level of radioactivity shows a sharp initial drop corresponding to the initial rise in radioactivity in the thyroid area. After a period of low activity, the level rises rather sharply to a small peak, drops off again, and rises gradually over the hours to a point where it begins to level off. The only deviation from this general pattern is that of one immature bird, No. 97 (Figure 3), where the initial drop is interrupted by a small rise in radioactivity.

The thyroid and blood curves may be summarized as follows: From 0 to 17 hours after injection there is a continuing rise in the level of radioactivity of the thyroid area, as the glands assimilate the radioiodine from the bloodstream. Correspondingly, the blood level of radioiodine drops. This is followed by a period in which the thyroid level of radioiodine is relatively high and the blood level is relatively low. At 39 to 43 hours, we observe what is probably evidence of the first appreciable secretion of radioiodine from the thyroid back into the bloodstream. In some cases, this is followed by a corresponding drop in the level of radioiodine in the thyroid area. After approximately 43 hours, the thyroid level of radioiodine gradually drops off, accompanied by a gradual rise and leveling off of the amount of radioiodine in the blood.

Discussion.—Even with the limits placed on the accuracy of the number of counts per minute, it is evident that the birds in Series 3 possessed radioactivity records much higher than those of Series 2. This may be an expression of 1) some phase of a seasonal fluctuation; 2) individual variability; or 3) a difference in the length of time the birds were held before injection. Since the figures in this report have not been scaled to allow for seasonal changes, it is not known whether a difference of 20,000 CPM would represent a small fluctuation or one so large that it would exclude this first possibility. The overlap indicated in the records of birds No. 92 and No. 98 suggests the second possibility. However, the chance that two groups selected at random would have a mean difference of the magnitude obtained would seem to be rather small. Further work will be necessary to clear up the questions raised by these three points.

Mature and Immature Birds.—There appears to be no appreciable difference in records between immature and mature birds, except for the minor difference in blood curves, noted above.

Advantage and Disadvantages of this Method.—The present method of recording the uptake and retention of iodine 131 permits the birds tested to remain alive, and thus the cycle of thyroid activity could be charted for the same bird throughout the year—a procedure that has formerly been impossible. The method also provides a more detailed scale of measurements for quantitative analyses of thyroid activity.

One possible disadvantage of this method may be the effects of capture on the activity of the thyroids of the birds tested.

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SUMMARY

White-crowned Sparrows and Brown Towhees were injected with iodine 131 and the radioactivity of the thyroid area was measured,

as well as the radioactivity of blood samples of 7 of the towhees, at given intervals after injection. All measurements were made on living birds. Peak radioactivity occurred in the thyroid area of the majority of the towhees about 17 hours after injection, at a time corresponding to the lowest level of radioactivity in the blood. Peak radioactivity occurred in the white-crowns some 2 hours earlier. There appeared to be no appreciable difference between the records of mature and immature individuals. The quantitative aspects of radioactivity records are briefly discussed. Advantages and applications of this method of measurement of live birds are also presented.

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