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Method for Determining Completeness of Thyroidectomy Using Radioactive Iodine.*

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It has been shown that radioactive iodine injected into or ingested by experimental animals is concentrated in the thyroid gland, and that it is possible to obtain evidence of its presence using either radioautographic^{1, 2} or electroscopic methods.³ It was thought reasonable to attempt to determine the presence or absence of thyroid tissue in thyroidectomized animals employing a sensitive indicator such as the X-ray film. The only valid method for determining completeness of thyroidectomy at the present time consists in producing complete serial sections of the neck region. In fact, in order to prove completeness of thyroidectomy, it is necessary to section the entire path of development of the thyroid gland from the base of the tongue down to and including trachea, thymus, great vessels and heart. Serial sectioning of this amount of tissue involves a disproportionate amount of time and labor.

To test the validity of a search for thyroid tissue using radioactive iodine, the following experiment was carried out. Twelve female rats of the Long-Evans strain thyroidectomized at the age of 70 days were injected at the age of 133 days with a solution containing radioactive iodine.[†] A normal, unoperated animal was included for purposes of control. Oxygen consumption tests were carried out shortly before autopsy.[‡] In those cases in which the oxygen consumption was greater than that expected of a completely thyroidectomized rat, the determinations were repeated. The lower figure is reported in each case.

The method employed for obtaining the radioautographs was as

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¹ Hamilton, J. G., Soley, M. H., and Eichorn, K. B., *Univ. of Calif. Publ. Pharm.*, 1940, **1**, 339.

² Gorbman, A., and Evans, H. M., *Proc. Soc. Exp. Biol. and Med.*, 1941, **47**, 103.

³ Perlman, I., Chaikoff, I. L., and Morton, M. E., *J. Biol. Chem.*, 1941, **139**, 433.

[†] The writer is indebted to the Radiation Laboratory of the University of California, and especially to Dr. J. G. Hamilton, for the supply of radioactive iodine (half life of 8 days) used in this work.

[‡] Oxygen consumption tests were very kindly conducted by Mr. Virgil Herring.

follows. The animals were injected subcutaneously 44 hours before autopsy with an aqueous solution (4 cc) of radioactive iodine, as the sodium salt, which had an activity of approximately 2 microcuries per cc. At autopsy, the neck region was exposed, the entire region of trachea and oesophagus bordering the normal site of thyroid tissue was dissected out *in toto* after careful inspection through the binocular dissecting microscope to determine the presence of gross fragments of thyroid which might have been left behind at operation. This tissue was fixed in 10% formalin. A further mass of tissue extending from the base of the tongue to the heart was dissected out and flattened on filter paper in the fresh state, and frozen in the cold room. This mass of tissue included the remaining trachea, hyoid region, base of the tongue, thymus, heart and great vessels, oesophagus, and muscles bordering the trachea. The separate pieces of tissue were mounted upon filter paper, covered with a sheet of thin cellophane, the whole being placed in an X-ray cassette of suitable size. A sensitive "non-screen" X-ray film was placed in direct contact with the cellophane covering the tissues, and the cassette was closed. The cassette with contained materials was kept in the cold room if fresh tissue was being studied. Exposures made over periods of duration of 1½ to 36 hours gave adequate evidence of radioactivity in the whole tissue mounts, not only in preparations in which the thyroid gland was present (normal control), and in grossly incomplete operations, but also in some of the animals which were judged to be completely thyroidectomized on the basis of gross inspection through the binocular microscope. As a further check on the amount of radioactivity present in some of the "incomplete" operations, the activity of certain of the iodine concentrating fragments was determined, employing the Geiger-Müller counter.[§] The results are summarized in Table I. Four methods of ascertaining completeness are listed. These are: weight gain after operation, oxygen consumption tests, gross inspection and presence of iodine concentrating tissue as determined by the radioautograph.

Several points are outstanding in the table. Approximately 50% of the animals which showed no visible evidence of thyroid tissue remaining in the neck upon binocular examination gave evidence of the presence of iodine concentrating tissue in the radioautograph. Gross examination was certainly not an adequate test of the completeness of thyroidectomy in this series. Secondly, in this series, it will be noted that a low oxygen consumption is not incompatible with the presence of iodine concentrating tissue in the neck (pre-

[§] These determinations were kindly made by Miss Helen Fahl.

TABLE I.
Comparison of Criteria for Determining Completeness of Thyroidectomy.

Thyroidectomized animal, No.	Final body wt, g	Post. body wt gain, g	Gross inspection	Oxygen consumption	Radio-autograph	Radio-activity
14	180	32	Complete*	105†	Incomplete*	562‡
15	203	15	"	129	"	1122
16	223	11	"	107	Complete	—
17	186	15	"	102	"	—
18	191	37	"	114	Incomplete	3818
19	192	21	"	131	"	—
20	183	—3	"	129	Complete	—
21	193	7	"	105	"	—
22	162	—3	"	131	Incomplete	—
23	180	35	Incomplete	143	"	2474
24	180	4	Complete	116	"	—
25	207	43	"	114	Complete	—
Normal control 26	189	48	Normal	169	Normal	200,000

*The term "incomplete" as employed above denotes the presence of thyroid tissue in supposedly completely thyroidectomized animals as determined by gross examination through the binocular microscope, or denotes the presence of iodine concentrating tissue as determined by the radioautograph.

†Oxygen consumption measured by the method of Haldane. Figures represent liters oxygen/m²/24 hrs.

‡Measurements made on the Geiger-Müller counter in counts/min. Figure for the normal control obtained by electroscope measurement.

sumably thyroid tissue). On the other hand, an animal with an oxygen consumption relatively high (repeated determinations) may show no evidence of iodine concentrating tissue in the entire neck region. This series of animals is too small to draw any definite conclusions as to the relationship between the oxygen consumption and the presence or absence of iodine concentrating tissue after thyroidectomy. This point, however, does merit further attention.

It is of interest that radioautographs of the neck tissues (other than those in the immediate normal location of the thyroid gland) showed no evidence of the presence of aberrant iodine concentrating tissue in this series. That aberrant thyroid tissues may exist is shown by the work of Erdheim⁴ for the rat and Kampmeier⁵ for the dog and other animals. As has been shown,² radioactive iodine may be present in other tissues than the thyroid. Thus in this series, muscle, heart, thymus, trachea and oesophagus all gave positive images on the X-ray film. In no case, however, did the intensity of this image compare with that cast by iodine concentrating tissue in the region of the thyroid gland. The possibility of self-absorption in these tissues was tentatively ruled out by demonstrating positive trans-

⁴ Erdheim, J., *Anat. Anz.*, 1906, **29**, 609.

⁵ Kampmeier, O. F., *Univ. of Ill. Bull.*, 1937, **35**, No. 12.

mission through a thick mass of tissues of the radiation from a normal thyroid and also from that animal's thyroid region (incomplete thyroidectomy) showing the smallest concentration of radioactive iodine.

The above evidence suggests the possibility of carrying out the same type of search for radioactive iodine in the thyroid employing the electroscope or Geiger counter, using some other tissue as a control for the amount of radioactive iodine present in tissues other than the thyroid gland. Some such procedure for locating aberrant or incompletely removed thyroid tissue is of great importance in any study in which the results depend on showing the complete absence of thyroid tissue in the thyroidectomized animal. It is of interest to note that the sizes of the images cast on the X-ray film by the iodine concentrating tissues are of the same order of magnitude roughly as the determinations on the Geiger-Müller counter. If there is a direct proportion between the amount of radioactive iodine stored in various sized fragments of thyroid gland as compared with the normal, then it would be possible to estimate roughly the amount of gland remaining in the thyroidectomized animal. Thus the fragment in animal No. 14 would be approximately 1/400 the size of the thyroid of the normal control animal.

It has here been assumed that the iodine-concentrating tissue determined by the radioautographic method represents thyroid tissue. Further studies are intended to correlate histological studies with ability to store iodine. The criticism may be made that absence of iodine concentrating tissue does not rule out the possibility of the presence of thyroid tissue. To the best of available knowledge, thyroid is the only tissue able to concentrate iodine in this particular fashion. Further it has not been shown that the thyroid gland or fragments thereof will fail to concentrate such injected radioactive iodine.

Summary. A simple and rapid method is suggested for ascertaining the presence or absence of iodine concentrating tissue (thyroid gland) in thyroidectomized animals. Injected radioactive iodine is assayed by radioautography of the entire neck region, either in the fresh or the fixed state.