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RADIOACTIVE IODINE AS AN INDICATOR IN THYROID PHYSIOLOGY

IODINE COLLECTION BY NORMAL AND HYPERPLASTIC THYROIDS IN RABBITS[1]

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In previous papers (1, 2) we have described the techniques and advantages of the use of radioactive isotopes of iodine as indicators in the study of iodine distribution and have reported preliminary results obtained with this method. This paper is concerned with the detailed results of extended experiments on normal rabbits and on rabbits which had received previous treatment designed to influence the physiologic state of the thyroid.

The majority of the experiments were conducted with the use of the radioactive isotope of iodine of mass 128, which has a half period of 25 minutes. We have also used a few samples of radioactive iodine with the half periods of 12.5 hours, 8 days and 13 days (mass numbers 130, 131 and 126 respectively) (3).

Purpose of experiments. It was the purpose of these experiments to investigate the collection of iodine by normal and hyperplastic thyroid glands, in order to establish the normal and pathological behavior toward iodine under various circumstances, and in order to determine the conditions under which it might be possible to use radioactive iodine to administer internal irradiation of the thyroid. The experiments have therefore been concerned with the measurement of the percentage collection of known doses of labelled iodine by the thyroid, as a function of time of collection, quantity of iodine injected, previous history of iodine treatment, thyrotropic hormone administration, cyanide injection, cabbage diet, sex, pregnancy, and certain combinations of these factors.

PROCEDURES. The iodine was usually administered intravenously in the form of sodium iodide obtained by dissolving labelled silver iodide in sodium thiosulphate (2). In a few experiments in which long collection times were possible because of the availability of long-period iodine iso-

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topes, subcutaneous injections were made. Animals were sacrificed by etherization, since the available radioactivities necessitated the removal of the thyroid for measurements.

The dosages of iodine administered (except in some preliminary work) were adjusted to be proportional to the weight of the animal, 2 kgm. being taken as a standard. Dosages below 0.5 mgm. were not so adjusted, however, it being of interest to determine the collection of the smallest available quantities of iodine. The range of dosages used was from less than 0.1 mgm. to 100 mgm.

In the present experiments, in addition to the information given by the gross appearance of the gland, we have used as a rough measure of the physiologic state of the thyroid the relative weight of the thyroid as compared with the body weight. It is well known that this is by no means a completely satisfactory indication of the physiologic state of the gland, but nevertheless, with a standard stimulus and large numbers of animals, it was possible to obtain statistically significant results.

For these purposes we have defined a quantity we call the thyroid index, as follows:

$$\text{Thyroid index (T.I.)} = \frac{\text{Weight of thyroid in milligrams}}{\text{Body weight in kilograms}}, \times 10$$

where the factor of 10 is introduced to give a convenient numerical result. For the normal animals in our colonies, the thyroid index of almost all animals ranged between 5.5 and 8.0. (See fig. 1.) In hyperplastic animals the index ordinarily ranges between 8.0 and 15.0, the highest value observed in our colony being 33.5 for an animal on an exclusive cabbage diet for 2 months.

In measuring thyroid iodine collection, we have introduced a quantity we call the thyroid concentration coefficient, in order to take into account the variation of size of thyroid glands among animals of the same weight. This quantity is defined by the relation

$$\text{Thyroid concentration coefficient (T.C.C.)} = \frac{\text{Thyroid iodine collection in per cent}, \times 1000}{\text{Thyroid index}}$$

The introduction of the factor 1000 has the result of making this quantity represent the concentration of iodine in the thyroid divided by the concentration in an equal weight of body tissue, assuming uniform distribution of the iodine. (Thus it is a measure of the affinity of the thyroid for iodine.)

This method of expressing the iodine collected by the thyroid is particularly useful because it gives directly the concentrating power of the thyroid as compared with the rest of the body. In some preliminary work to determine the approximate character of the iodine collection of various stimulated glands, no indices were constructed, and the data are accordingly presented in terms of percentage. A direct comparison between percentage collection and thyroid concentration coefficient may be obtained from figures 4 and 6. Previously published data (1) have shown that the collection of iodine by the thyroid is much greater than by other organs.

All animals (except those on a cabbage diet) were maintained on a uniform rabbit chow diet, in a room kept free of any gross contamination with iodine. Thyrotropic hormone, cyanide and iodine other than the

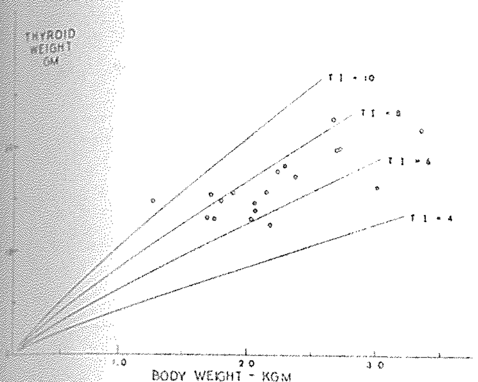

Fig. 1

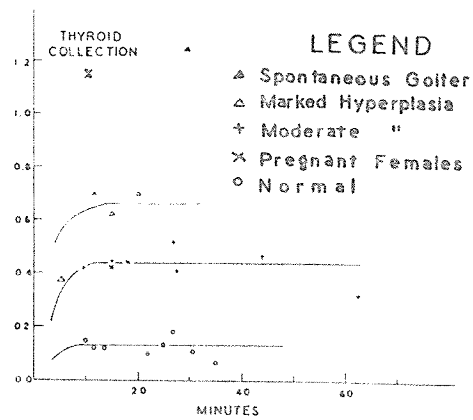

Fig. 2

Fig. 1. The variation of thyroid weight with body weight for some of the normal animals used. The radial lines are lines of constant thyroid index.

Fig. 2. Preliminary survey of the percentage collection of the thyroid in various time intervals after injection of a 10 mgm. dose of iodine. The data show the marked dependence upon the functional state of the thyroid. No precautions were taken to adjust the size of injection to the size of the animal, and the degree of hyperplasia was judged from the appearance of the gland alone.

labelled iodine were injected subcutaneously or intramuscularly. Animals on a cabbage diet were fed exclusively on cabbage, no water being given.

Cyanide-injected animals were maintained on a normal diet, and were given daily injections of 0.1 cc. of methyl cyanide daily for 2 to 4 weeks. Animals that were given 0.25 cc. daily exhibited a very high mortality rate without a great change in thyroid response.

Several different preparations of thyrotropic hormone have been used during the course of the experiments. These include suspensions of fresh beef anterior pituitary, of desiccated anterior pituitary powder supplied by Armour and Co., material prepared according to the technique of

Lambie and Trikojus (4) (in both the soluble and insoluble forms), and a thyrotropic extract prepared and kindly furnished to us by Dr. Oliver Kamm of Parke, Davis and Company. Of all these the last named was found to be the most active and satisfactory preparation. With this material 50 to 100 per cent increase of thyroid size in normal adult male rabbits may be obtained with 2 successive daily injections, corresponding to a nominal daily dose of 25 guinea-pig units. In early experiments, Ayerst, McKenna and Harrison thyrotropic preparations were used, but were found to be inactive in male rabbits, in dosages up to 10 cc.

Pretreatment with iodine. A series of experiments were undertaken to determine the effect upon the collection of a labelled dose of iodine of

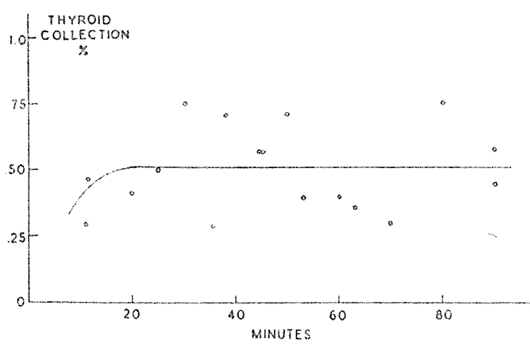

Fig. 3

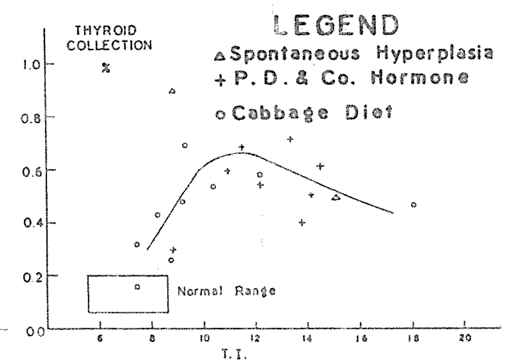

Fig. 4

Fig. 3. Percentage collection of the thyroid of normal males of injections of between 0.1 and 0.4 mgm. of iodine. No increase of collection over that of the first 10 minutes is observed for intervals up to an hour and a half.

Fig. 4. Percentage collection of hyperplastic thyroid glands of male animals. Hyperplasia induced by cabbage diet, by injection with Parke, Davis and Company thyrotropic hormone, and of spontaneous occurrence. Injection 5 mgm. per standard 2 kgm. animal. Collection time 15 minutes. The differently treated animals show experimentally indistinguishable responses, with a maximum collection at T. I. ca. 11 or 12. These animals are referred to as type 1.

previous iodine treatment. In these experiments the labelled dose of iodine was always 5 mgm., while the amount used in pretreatment was varied from 5 mgm. to 100. The time elapsing between pretreatment and injection of the labelled dose of iodine was varied from several minutes to several days.

Experiments with more than one radioactive isotope. In some of these experiments it was possible to label the dose used for pretreatment as well as the succeeding dose, using a different radioactive isotope for each dose, and distinguishing between the 2 or more radioactivities found in the thyroid by following the decay curve of the total radioactivity in the gland, and analyzing it into its several components. In the analogy of labelling, this corresponds to the use of labels of different colors for the different doses.

Results. Most of our data are presented in graphical form, in figures 1 to 10.

Using the long-period isotopes referred to above, we have obtained some data on the collection of single doses of iodine in hyperplastic thyroids in periods up to 8 days. The results show that the collection in type 1 animals (see figs. 4–6 for explanation of different types) tends to increase until the total collection has reached the value of about 40 to 50 micrograms, and then does not increase further. The length of time this takes

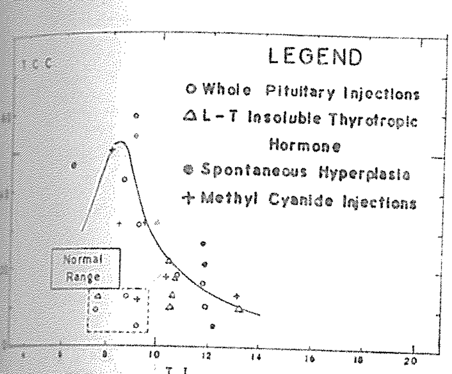

Fig. 5

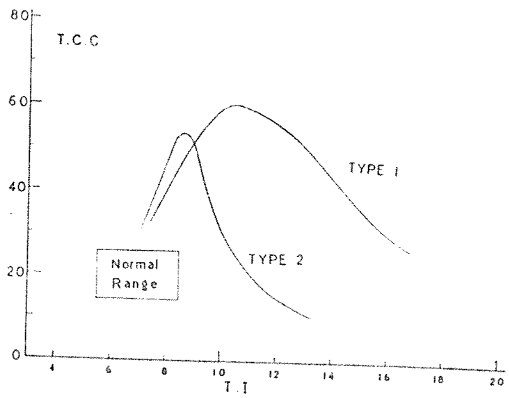

Fig. 6

Fig. 5. Thyroid collection coefficients of male animals treated as follows: saline suspensions of fresh beef anterior pituitary, insoluble thyrotropic hormone prepared according to the method of Lambie and Trikojus, methyl cyanide injected, and untreated animals with spontaneous hyperplasia. Injection 5 mgm. per standard 2-kgm. animal. Collection time 15 minutes. For a discussion of the points in the dotted rectangle, which were not taken into account in drawing the curve, see the section entitled "Discussion." These animals are referred to as type 2.

Fig. 6. Thyroid concentration coefficients for the data of figures 4 and 5.

depends mainly upon the total quantity of iodine injected. From figure 9 this may occur in as little as 15 minutes. The smallest quantities of long-period labelled iodine injected have been of the order of 1.0 to 1.5 mgm. The thyroid collection of this quantity may go as high as 6 per cent within a day, in animals on a cabbage diet. This is to be compared with collections of as much as 6 per cent within 15 minutes with injections of ca. 0.2 mgm.

In an extension of the results given in figure 10, 6 animals have been injected with 2 successive labelled doses of iodine and the collection from each dose measured. The results seem to show that if a moderately large collection occurs from the first dose, the collection of the second dose is inhibited. Thus an animal which in 19 hours collected 53 micrograms from a 4 mgm. injection collected less than 4 micrograms from the subsequent 5 mgm. injection. On the other hand, an animal which had collected only 20 micrograms from a 5.5 mgm. injection in 31 hours, collected 7 micrograms from the subsequent 5 mgm. injection. The collection time of the second injection was always 15 minutes. In one animal whose collection time was 8 days, the collection was not much more than it would have been in 15 minutes.

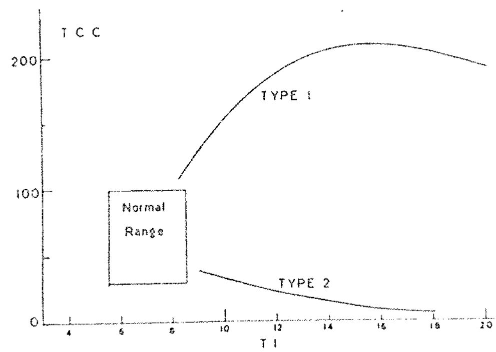

Fig. 7

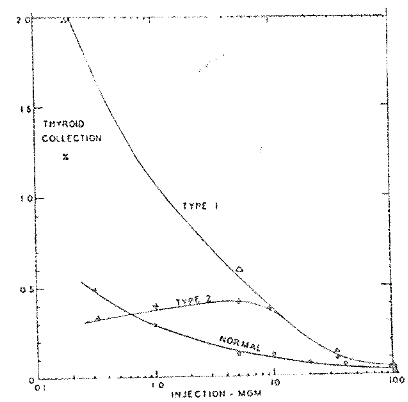

Fig. 8

Fig. 7. Same type of data as figure 6 for injections of 0.2 to 0.5 mgm. per standard 2-kgm. animal. In type 2 animals are now included some which received a dilute acetic acid suspension of Armour's desiccated pituitary tablets, and some receiving the soluble thyrotropic hormone prepared according to the method of Lambie and Trikojus (4). The curve for type 1 animals is only approximate in character, since the scattering of the values of the thyroid collection coefficient is great, the values ranging from 100 to 500. The number of animals used was 12 type 1, 20 type 2.

Fig. 8. Variation of the percentage iodine collection of the thyroid in 15 minutes with the quantity of iodine injected. The curves for hyperplastic animals are for average thyroid index 10. Total number of animals used: 39 type 1, 68 type 2, 69 normal.

In most of the experiments on pretreatment with iodine, the premedication was accomplished with ordinary iodine. The effects of premedication depend upon a large number of other factors in animals with hyperplastic thyroids. The subsequent collection appears to depend not only upon the quantity of the premedicating dose, but also upon the thyroid index of the animals, the type of thyroid stimulation, and to some extent the time elapsing between the premedicating dose and the labelled dose. In normal animals a dose of about 50 mgm. is necessary to reduce the collection of the labelled dose to half the value expected of a normal untreated animal. In the case of type 1 animals, only 8 mgm. will do the same within 4 to 6 hours. (See fig. 10.)

A number of control experiments with radioactive bromine have been performed. The thyroid collection in both normal and hyperplastic animals is less than one-tenth of that from the same quantity of iodine, and the presence of the bromine could be demonstrated in the cerebrum within 15 minutes, in contra-distinction to the behaviour of the iodine, which could not be detected there.

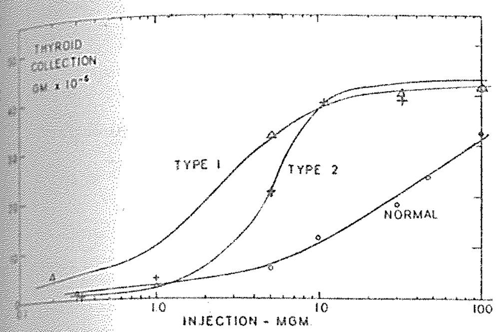

Fig. 9

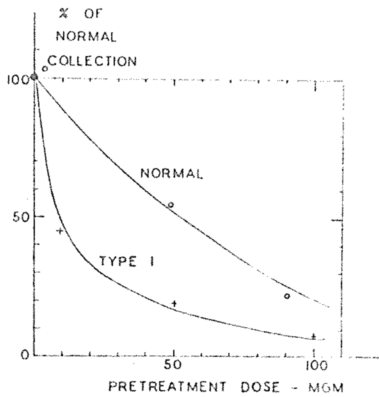

Fig. 10

Fig. 9. Total iodine collection of the thyroid from different injections in 15 minutes. The data are the same as those plotted in figure 8.

Fig. 10. The effect of iodine premedication upon the collection of a subsequent 5 mgm. labelled iodine injection in 15 minutes. The collection is given in terms of the collection without previous treatment, which is set at 100 per cent. The time elapsing between the two doses is between one hour and five days, in normal animals. The curve for type 1 animals is for thyroid index 10, and for an interval of time between the premedicating dose and the labelled dose of 4 to 6 hours. The number of animals used was 9 normal, 12 type 1.

Discussion of results. From figures 2 and 3 we see that the collection of iodine in both the normal and hyperplastic gland reaches a value within 10 minutes which is not thereafter exceeded for at least 1.5 hours. This has been verified for all the values of injection for which experimental points are plotted in figure 8. In a few cases, collection times of less than 5 minutes have been used; collection has been detected in animals that died within 2 minutes of injection. It appears, therefore that the *initial collection* (i.e., the collection within 1.5 hours) *is taken up in a time determined mainly by the time required for the injected material to reach the thyroid.* In the few experiments performed with long-period iodine, we have found that in general the increase of collection, if any, is comparatively slow following the initial collection.

The percentage of iodine collected from any given injection by the thyroid is definitely dependent upon the dosage, in all types of thyroids.

Figure 8 shows this dependence. For normal thyroids, *the percentage collection increases as the dosage is decreased*. The total quantity of iodine so collected, however, is small even when the injected dose is 10 mgm. or more. On the other hand, the efficiency of collecting a dose of 0.1 mgm. is much higher (see fig. 8). In terms of the concentration coefficient, the T.C.C. at 0.1 mgm. is about 80 for normal glands, while for an injection of 100 mgm. it may be as low as 2, or less. This relatively large percentage uptake from small injections seems qualitatively consistent with the ability of the normal gland to maintain itself upon the small quantities of iodine normally found in the diet.

From figures 4, 5, and 6, it appears that under various conditions of stimulation the thyroid can respond in (at least) two different ways, with respect to the iodine collection of doses of 5 mgm. In one type, which we have called type 1, the T.C.C. is always higher than normal, and in the other, type 2, the T.C.C. is significantly lower, and falls below normal for large values of the T.I. (In fact, for several of the animals—those in the dotted rectangle in fig. 5—the T.C.C. is below normal although the gland has increased only slightly in size. This will be discussed a little later on.) The two different types are defined by the character of their pretreatment, except in the case of animals with spontaneous hyperplasia, which were found to fall in both categories. There is for each type a maximum T.C.C. at a particular value of T.I. It is quite striking that glands of the same size, e.g., T.I. 13, do not exhibit the same iodine collection, although their gross appearances are similar, both appearing hyperplastic. The decrease of T.C.C. for thyroid indices larger than 10 can be interpreted in different ways. If we admit that the uptake of iodine under the conditions stated is a measure of the functional activity of the thyroid, then this decrease may be interpreted as due either to the existence of a threshold, or to functional exhaustion of the gland (or both). If we assume it to be due to a threshold, then we must suppose that in general the higher the thyroid index in type 2 animals, the higher the threshold for iodine uptake.

In this case we should expect the shape of the curves for type 2 animals to change, as the dosage of iodine is changed. Figure 7 shows the altered shape of the curves for very small injections. In this case, the type 1 animals follow the same sort of curve as in figures 4 and 6, and exhibit very high collections, reaching in certain cases as much as 6 per cent of the injection in 15 minutes. On the other hand, the type 2 animals always collect less than the normal at this injection level. This curve casts some light upon the animals referred to above, represented by the points in the dotted rectangle in figure 5.

These animals have thyroid indices only slightly greater than normal, but their collections are below normal even with a 5 mgm. injection. A similar curve at an injection of 10 mgm. does not show any such points. Therefore, we have drawn the curve in figure 5 through the upper points, considering these animals to have a threshold for iodine collection which is greater than the average at their thyroid index. In some of the animals which received very small injections, the collection of iodine by the thyroid was so small that it was undetectable (less than 0.05–0.1 per cent of the injection) and only maximum values for the iodine collection could be given. The contrast between the type 1 and type 2 animals is even more striking at this value of injection than at the higher injection value.

Figures 8 and 9 again show the threshold effect, which can best be discussed with reference to the latter. In this graph we see that the total iodine collected by type 2 glands of a given size (T.I. 10) is rather high and fairly constant for injections above 10 mgm., but that it drops sharply between 1 and 10 mgm., becoming consistently lower than the normal collection somewhere near 1 mgm. On comparison with the behavior of the type 1 animals it appears that this is quite definitely a threshold effect and perhaps even an "all-or-none" effect. The relatively gradual decrease in total iodine collection of the type 1 animals shows that if similar considerations apply to these glands, they do not begin to do so in the dosage range investigated.

The functional exhaustion involved in the type 2 animals is not a complete exhaustion, but a threshold effect, because at injections of 10 mgm. and higher values, the collection of these animals is above normal in all cases, and is indistinguishable from the behavior of the type 1 animals.

It is interesting to note that for large doses, the collection of iodine in both types of glands attains a nearly constant value at about 40 to 50 micrograms. If the wet weight of a rabbit thyroid is about 200 mgm., of which 60 per cent is water, this quantity of iodine represents a collection of 0.05 per cent of the dry weight, or a quantity of the same order of magnitude as the original iodine content of a hyperplastic gland. Thus the hyperplastic gland is capable of increasing its iodine content 100 per cent within a few minutes.

We do not wish to suggest that the two types of hyperplastic glands discussed here constitute strictly defined separate categories. The curves of figure 9 seem to indicate that perhaps there can be continuous gradations of all types between the limits plotted in the graph, and even outside these limits. Evidence for this is given by the shape of the curves giving the variation of collection with thyroid index, which indicate that the threshold for iodine collection is somewhat displaced for values of the thyroid index other than 10. It has, of course, been previously known that various types of thyroid hyperplasia exist which are functionally different. The dissimilarity in behavior toward iodine is another indication of this multiplicity.

We have so far assumed that the appearance of the labelled iodine in the thyroid after injection is actually due to addition of new iodine to the gland, and not to simple exchange of the iodine previously present with the labelled iodine. We have several reasons for supposing this to be the case, although even if it were not, the iodine exchange could be used as an indication of the thyroid function, or to introduce internal irradiation, just as well as iodine collection. Our reasons for believing that we are dealing with collection rather than exchange are the following: hyperplastic glands collect more iodine than normal glands at some injection values, less at others. The amount exchanged by a gland ought ultimately to be independent of dosage, and to depend mainly upon the iodine content of the gland. This does not appear to be the case. Furthermore, the collection of the second dose of iodine is almost always much less than the collection of the first dose. The amount exchanged ought to be independent of whether the dose is the first or any other in order.

In view of the fact that there is a difference in iodine collection at the same thyroid index, at low injection values, in animals treated with Parke-Davis thyrotropic hormone and animals treated with other thyrotropic hormone preparations, including the saline suspension of fresh beef anterior pituitary, it appears that there may be separate factors producing growth and functional stimulation with respect to iodine collection, respectively. This is in accord with the clinical observation that the size of the thyroid, taken by itself, is not indicative of the functional state.

The difference between the behavior of cabbage diet animals and animals injected with methyl cyanide is worth noting, in view of the supposed cyanide nature of the goitrogenic substance in cabbage (5).

Radiation therapy with radioactive iodine. From the data on iodine collection that we have obtained, and the known energy of the radiations from the various radioactive isotopes of iodine, it is possible to calculate how strong the radioactivity of an administered dose of radioactive iodine must be in order to give any desired amount of radiation within the thyroid. Since the thyroid concentration coefficient may be as much as several hundred, it is easy to see that the ratio of thyroid dosage to the dosage of other tissues may be made just as high, thus giving a very large safety factor for such irradiations.

If we assume that it is desired to administer such an amount of radioactive iodine as to yield, in decaying to zero activity, a dose of 100 r within the thyroid, then the amount of initial activity required per gram of thyroid tissue has been calculated (6) to be:

For 25 min I: 100 r = 0.1 millicurie per gram thyroid
For 8 day I: 100 r = 0.6 microcurie per gram thyroid.

These activities are very different from each other, and we must now consider the Bunsen-Roscoe reciprocity law. It is a question still under debate as to whether a weak source supplying radiation for a long time will have the same effect as a strong source supplying the same total radiation. The initial rates of irradiation for the above activities are respectively:

25 min. I : 2.7 r. per minute
8 day I : 0.4 r. per hour

Normal tissues tolerate safely a dosage of 0.1 r. per day for indefinite periods; it is therefore questionable whether the 8-day iodine of the strength calculated above is supplying its radiation fast enough to have the desired effects. This is something to be decided by experiment. On the other hand, the short-period iodine will approximate quite well the short intense dosage obtained in x-ray therapy.

Considering only the 25-minute period, then, the necessary activity per gram of tissue is 0.1 mC., for an initial intensity of ca. 3 r. per minute. If we assume a thyroid to collect 1 per cent of the administered iodine, as the results of these experiments permit us to suppose likely, the injected dosage must then be 10 mC. per gram of tissue. For the rabbit, with a thyroid of ca. 0.2 gram, we must correct the value thus obtained for the "leakage" of radiation outside the thyroid tissue, since some beta rays can escape from the thyroid because of its relatively small size, and will dissipate part of their energy in the surrounding tissue. This will certainly be taken care of by adding 50 per cent to the required dosage. Thus, for the rabbit, we may calculate that the required dosage to provide 100 r. of radiation in the thyroid is about 3 mC. of the 25-minute isotope of iodine.

Since preliminary experiments show that similar collections may be obtained in human thyroids, then a 75 gram thyroid will require an administered dosage of ca. 750 mC.

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SUMMARY

Using radioactive iodine as an indicator, the quantity of iodine taken up by the thyroid of the rabbit under various circumstances was studied. After intravenous injection, the percentage collection from any given dose was found to reach a maximum within ten minutes, which was not greatly

exceeded for periods of collection as long as several days. The normal thyroid was found to collect up to 80 times the quantity to be expected from uniform diffusion into the general body tissues; the hyperplastic thyroid will collect up to several hundred times the quantity expected from uniform diffusion. The variation of this concentration with the injected dosage and the functional state of the gland was determined. The effect of pretreatment of the thyroid in various functional states, with iodine, on the collection of a subsequent dose of labelled iodine was measured.

In certain of these experiments, several differently labelled iodine injections were used on the same animal in order to determine the fate of the individual doses. This labelling was accomplished by using different radioactive isotopes.

From the data thus obtained, we have calculated the strength of samples of radioactive iodine, with which it will be possible to administer internal irradiation of the thyroid for therapeutic purposes.

Clinical implications of the results are discussed.

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