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IN THE LABORATORY

Methods for Labeling Thyroxine With Radioactive Iodine[1]

EARL FRIEDEN, MORTIMER B. LIPSETT, and RICHARD J. WINZLER

Department of Biochemistry and Nutrition, University of Southern California School of Medicine

In order to study the metabolism of thyroxine it became necessary to consider the methods for labeling thyroxine with I^{131} and the positions of the radioactive iodine atoms. We wish to report on two methods that we have used to prepare radiothyroxine of high activity.

Radiothyroxine has been prepared by Horeau and Suë (*3*) from 3,5-diiodothyronine using the method of Harington and Barger (*2*), in which the iodinating agent is iodine in ammoniacal solution. It has been assumed that the compound so prepared is 3′,5′-di-I^{131},3,5,di-I^{127} thyronine. This supposition is of some importance in experiments dealing with the fate of injected thyroxine, since differences undoubtedly exist in the reactivities of the iodines of the two rings. Thus, deiodination *in vivo* may proceed preferentially at one or the other rings, and a knowledge of the position of the iodine atoms is pertinent.

The total synthesis of thyroxine by the classical method of Harington and Barger (*2*) does not offer a practical route for the preparation of the tetra-I^{131}-labeled thyroxine. This is due to the relatively short half-lives of the available iodine isotopes and an over-all yield of less than 5% in the 10-step procedure.

Labeled thyroxine has also been isolated after the injection of I^{131} into animals with functioning thyroid glands as well as after incubation of thyroid slices with I^{131} (Morton, Chaikoff, *et al.*, *5*). However, these biosynthetic methods are not practical for the preparation of radiothyroxine of high specific activity in the amounts needed for biological work. It is likely that thyroxine so prepared contains I^{131} distributed among the four positions.

A simple and convenient technique for preparing radiothyroxine was suggested by the work of Miller, *et al.* (*4*), who studied the exchange reactions of diiodotyrosine with iodine and iodide ion. They found that at pH 5 and 50° C iodine exchanged almost completely with the iodine of diiodotyrosine in 90 min.

We have similarly prepared radioactive thyroxine by an exchange reaction. In a typical experiment 5 mg of *dl*-thyroxine was introduced into 25 ml of a 9:1 butanol-water mixture at pH 5 containing 0.10 mg of I^{127} and 10 μc of I^{131} as the iodides. After being refluxed for 12 hrs, the mixture was cooled and any undissolved thyroxine removed by filtration and thoroughly washed. The remaining butanol solution was washed to remove inorganic iodide and the butanol removed *in vacuo*. The thyroxine fractions were then recrystallized from boiling 0.1 N sodium carbonate solution. In several runs, up to 30% of the radioactive iodine could be recovered in the recrystallized thyroxine. This, of course, indicates that under these conditions complete exchange was not achieved. However, with this exchange reaction, using an initial radioactivity of 10 mc of I^{131} in the solution, it should be possible to prepare 5-mg quantities of radiothyroxine with an activity of greater than 10^6 disintegrations/μg/min and thus study the metabolism of thyroxine when given at *physiological levels.*

Since we have no evidence as to whether or not all the iodine atoms in thyroxine are involved in this exchange, data obtained with this radiothyroxine may be subject to the limitation discussed above for the preparation of radiothyroxine from 3,5-diiodothyronine. The question of which iodine atoms are involved in this exchange will be difficult to solve. Previous methods employed by Har-

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ington (*1*) to split the diphenyl ether linkage would result in the simultaneous elimination of the iodine. A more fruitful approach would be study of the rates of exchange of the compounds 3,5-diiodothyronine and 3′,5′-diiodothyronine with I^{131}.

A convenient method of preparing thyroxine with I^{131} of identical specific activity in all four positions is through the *in vitro* iodination of certain tyrosine-containing proteins. We have prepared radiothyroxine in this way, using the procedures described by Reineke and Turner (*7*). Fifty gm of casein was iodinated using 12 gm of powdered iodine containing 10 mc of I^{131}. After hydrolysis of the iodinated casein with barium hydroxide, *dl*-thyroxine with an activity of about 10^4 disintegrations/μg/min was isolated in the usual way.

Another possible method of labeling all iodine atoms in thyroxine is the direct *in vitro* conversion of diiodotyrosine to thyroxine, as first reported by von Mutzenbecher (*6*). The low yields of thyroxine obtained make this method less desirable for the preparation of radiothyroxine than the other methods discussed.

The ease of preparation of radiothyroxine of high specific activity should facilitate further research into the metabolism of thyroxine administered at physiological levels.

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A Convenient Plant Pollinating Kit

C. A. Schroeder

Division of Subtropical Horticulture, University of California, Los Angeles

During a series of hand-pollination experiments on the cherimoya considerable inconvenience and loss of time were experienced in preparing plant tags, transferring pollen, and entering field data in the notebook while using ordinary equipment. When large numbers of detailed, individually marked pollinations were made, considerable lost motion and time resulted from reaching into the shirt or trouser pocket for tags and pencil, picking up the pencil, which was dropped occasionally, transferring the pollen vials from pocket to hand, and finding some place to rest the field notebook while other operations were under way. Such losses were greatly accentuated when working on a ladder high in a tree. It was found very convenient to have all necessary "bookkeeping" equipment and other important experimental materials available and securely attached to a portable shelf or kit suspended by a cord around the neck.

The kit (Fig. 1) consists of a breastboard 6″ × 8″ and a shelf 6″ × 6″ made of ¼″ plywood. The breastboard provides stability for the apparatus and prevents twisting and

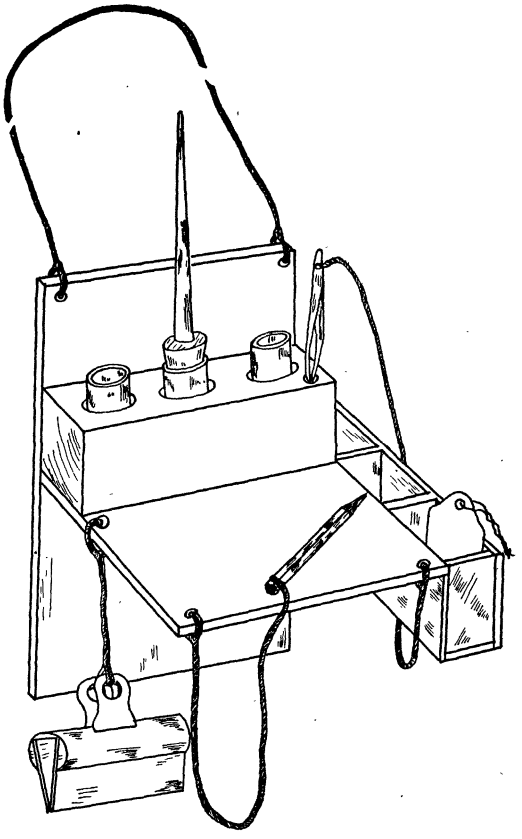

Fig. 1

fouling when working among tree branches. The shelf functions as a small desk so that a convenient and smooth writing surface is available at all times. A small block 1½″ × 1½″ × 6″ has holes drilled to receive small vials of pollen. A series of small wooden pockets are attached to one side of the desk to hold paper or wooden plant tags. Small holes drilled at the desk corners provide for attachment of a pencil, forceps, and a large spring clip from which the field notebook is suspended. The brush used for pollination work is mounted in a cork and kept in one of the pollen vials.

The compactness and convenience of the kit is self-evident. All items except the plant tags and brush are secured to the desk and cannot be dropped. When suspended from a cord passed around the neck, the kit is always in a vertical position and immediately in front of the operator in whatever position he may be. It also allows the worker's hands to be free at all times between operations.

It is thought that the basic idea may be of interest to those who have to make detailed recordings of specific pollination or other similar operations in the field.