occurring non-toxigenic strains of *C. diphtheriae* will induce toxigenicity in certain non-toxigenic strains of *C. diphtheriae* as readily as will phages derived from naturally-occurring toxigenic strains.

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Tissue Conversion of Thyroxine to Triiodothyronine.* (21949)

JOHN R. HOGNESS, MARGARET BERG, PAUL P. VANARSDEL, JR., AND ROBERT H. WILLIAMS.

From the Department of Medicine, University of Washington School of Medicine, Seattle.

The isolation of a new thyroxine-like compound from the thyroid and plasma by Gross and LeBlond(1,2) and the subsequent identification of this substance as 3,5,3′-triiodothyronine by Gross and Pitt-Rivers(3,4), and Roche, Lissitsky and Michel(5) have stimulated a great deal of investigative work. Triiodothyronine has been shown to be about 5 times as potent as thyroxine in preventing thiouracil-induced goiter in rats(6,7,8) and to produce a much more rapid elevation in the oxygen consumption of hypothyroid animals and man than does thyroxine(8-11). These findings, along with the earlier reports by Gross and LeBlond(1) of isolation of the I^{131}-triiodothyronine from tissues and plasma of thyroidectomized animals given I^{131}-labeled thyroxine, led Gross and Pitt-Rivers(6) to postulate that thyroxine is enzymatically deiodinated to triiodothyronine in the tissues and that triiodothyronine is the active form of the hormone at the tissue level. Although Albright, Larson and Trust(12) reported the conversion of thyroxine to triiodothyronine by kidney slices and MacLagan and Sprott(13) made similar observations using liver homogenates, Roche, Michel, Michel and Lissitzky (14) have been unable to demonstrate the deiodination of thyroxine to triiodothyronine *in vitro*. More recently Roche and Michel(15), on the basis of *in vivo* work with thyroidectomized animals, found that the thyroid is the sole important source of plasma triiodothyronine and that there was no triiodothyronine or its derivatives in the bile or urine of such animals after the injection of labeled thyroxine. Kalant, Lee, and Sellers(16) reported that triiodothyronine was the major radioactive compound found in the skeletal muscle of propylthiouracil-treated rats given I^{131}-labeled thyroxine but Taurog(17) stated that his group did not find consistent conversion of thyroxine to triiodothyronine in liver, kidney, spleen or diaphragm.

With these conflicting observations in mind, the following experiments were devised in an attempt to demonstrate the *in vivo* conversion of thyroxine to triiodothyronine by the rat.

Methods. (a) Thirty-three male Sprague-Dawley rats, weighing 250 to 300 g and maintained at 80°F and on standard Purina Laboratory Chow were given 10 μc (0.5 to 0.7 μg) of I^{131}-labeled thyroxine† intravenously. (The stock solution of thyroxine consisted of this hormone in 50% propylene glycol in water: aliquots were diluted with 0.9% saline before administration.) At intervals of 30 minutes, one, 3, 6, 12, and 24 hours after injection, liver, kidney, and skeletal muscle were removed. Nine rats were studied at the 30-minute point, 3 at one hour, 7 at 3 hours, 3 at 6 hours, 4 at 12 hours and 7 at 24 hours.

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† Obtained from Abbott Laboratories, Oak Ridge, Tenn.

These tissues were homogenized in NH_4Cl buffer (pH 8.4), boiled for 30 minutes, digested with crude trypsin for 20 hours and extracted 3 times with n-butanol. The butanol extract was then evaporated to dryness and the residue taken up in 2 to 3 ml of 0.5 N NaOH. Identical experiments were performed either using purified trypsin instead of crude trypsin or omitting trypsin digestion entirely with essentially the same results except that digestion with either purified or crude trypsin produced a slightly higher yield of butanol-extractable material. The material taken up in NaOH was then run through kieselguhr chromatographic columns according to the method of Gross and Pitt-Rivers(4)‡ and Braasch, Flock and Albert(18). Butanol containing 20% chloroform equilibrated with 0.5N NaOH was run through the column until thyroxine, triiodothyronine and iodide were eluted. The solvent system was then changed to 10% isopropyl alcohol in butanol for the purpose of recovering di- and monoiodotyrosine. The effluent was collected with a fraction collector and the radioactivity of each fraction was assayed with a scintillation detector. The results were expressed graphically, plotting counts per minute in the fraction versus fraction number. The relative amounts of all radioactive components recovered was determined roughly by comparing the areas under the peaks obtained. (b) An experiment using 16 rats was performed identical to that described above except that 10 μc (0.5 to 0.7 μg) of I^{131}-labeled triiodothyronine was injected into the animals. The animals were sacrificed 1, 3, 6 and 12 hours after the injection of triiodothyronine. Four animals comprised each group. (c) An experiment similar to that described under (a) was carried out in 16 animals 2 weeks after thyroidectomy. In this case 10 μc of thyroxine which had been re-purified by passage of the stock solution through the kieselguhr column was injected intravenously and the animals sacrificed 30 minutes after injection. Specimens of liver, kidney, and muscle were homogenized and

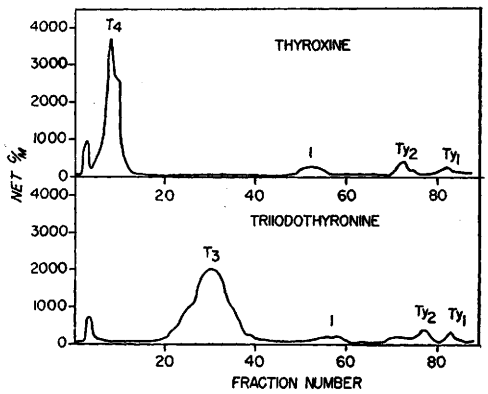


FIG. 1. Representative graphs of kieselguhr chromatographic analysis of liver, kidney and muscle at various time intervals after injection of (a) I^{131}-labeled thyroxine and (b) I^{131}-labeled triiodothyronine. T_4 indicates thyroxine; T_3, triiodothyronine; I, iodide; Ty_1, monoiodotyrosine, and Ty_2, diiodotyrosine.

treated as described above. Other specimens of liver and kidney were homogenized in sucrose solution at 4°C and separated, by means of differential ultracentrifugation(19), into nuclear, mitochondrial, microsomal, and residual protein fractions. The fractions thus obtained were treated as described in the initial part of the experiment and the extracts were subjected to kieselguhr chromatographic analysis.

Recovery studies with either labeled thyroxine or labeled triiodothyronine revealed that 98 to 101% of the radioactivity placed on the column was recovered in the effluent.

Results. Normal rats, whole tissues. Fig. 1 is representative of the results obtained in liver, kidney or muscle from intact normal rats following the administration of labeled thyroxine and triiodothyronine. The chromatographs were essentially alike at all time intervals studied. When thyroxine was administered the main compound recovered corresponded to thyroxine on chromatographic analysis, and in most instances no triiodothyronine was recovered. However, in 20% of the total number of chromatographic analyses, small triiodothyronine peaks were observed following the administration of thyroxine. Fig. 2 characterizes the findings in these instances. The triiodothyronine peak was present at all of the various time intervals following injection, including the 30-minute point

‡ The authors wish to express their gratitude to Dr. Jack Gross for his invaluable help and advice in the use of the kieselguhr chromatographic technic.

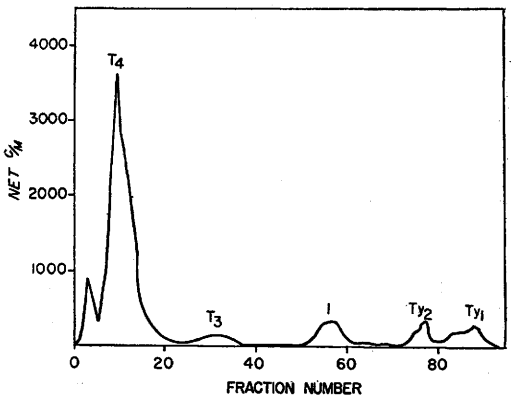


FIG. 2. Plot of radioactivity recovered from kieselguhr columns representative of instances (20% of total assays) where triiodothyronine was recovered from liver, kidney and muscle after injection of I^{131}-labeled thyroxine.

and, although recovered occasionally from all 3 tissues, it was found more frequently in liver and kidney than in muscle. When the approximate relative amount of radioactivity present in each of the chromatographic peaks was determined by measuring the area under each peak, the radioactivity corresponding to triiodothyronine never exceeded 1.5% of the total.

When triiodothyronine was administered, triiodothyronine was the main product of the column separation and no thyroxine was observed at any time. *Thyroidectomized animals*: In the 16 thyroidectomized animals injected with thyroxine and sacrificed 30 minutes later, essentially the same results were observed as in the normal animals. In approximately 20% of all analyses done on liver, kidney and muscle in these animals, small triiodothyronine peaks were detected, the traces of this substance occurring apparently at random in all 3 tissues. In no case did the triiodothyronine peak comprise more than 1.5% of the total radioactivity recovered.

Subcellular fractionations. Fig. 3 is illustrative of the observations made after thyroxine injection into thyroidectomized rats when subcellular fractions (microsomes, mitochondria, nuclei and residual protein) of liver and kidney were subjected to chromatographic separation. Similar findings occurred in the fractions of both liver and kidney. In cases where traces of triiodothyronine were found in the whole homogenates of these tissues, relatively greater amounts of this compound were isolated from some of the subcellular fractions. The greatest concentrations of triiodothyronine appeared in the microsomes and residual fraction of both liver and kidney, the amount of triiodothyronine running as high as 10.5% of the total radioactivity in the case of soluble protein fraction of the kidney. It was of great interest that even in some cases where no triiodothyronine was found in the chromatographic separation of whole homogenates, significant amounts of triiodothyronine were found in the subcellular fractions.

Discussion. By the method of kieselguhr chromatographic separation, we were able to recover triiodothyronine from whole rat tissues following the administration of thyroxine. Triiodothyronine was recovered from liver, kidney and muscle in about 20% of the chromatographic analyses made. When it was found, it was present in only small amounts, never exceeding 1.5% of the total radioactivity recovered. These findings are somewhat at variance with those of Kalant *et al.* with respect to the amounts of triiodothyronine recovered. If thyroxine is actually metabolized to triiodothyronine, and if the appear-

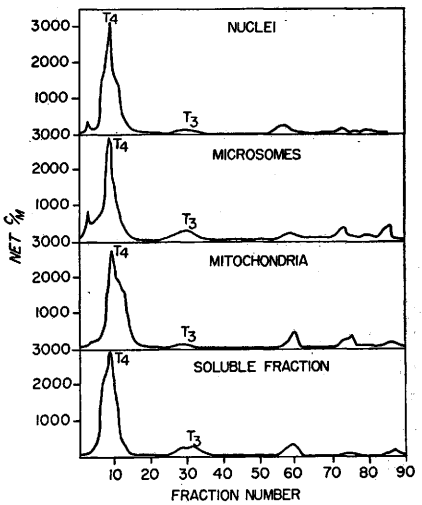
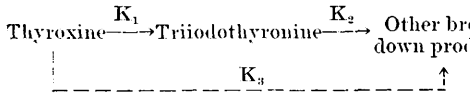


FIG. 3. Radioactivity in effluent from kieselguhr columns representative of findings in subcellular fractions of liver and kidney following injection of I^{131}-labeled thyroxine.

ance of triiodothyronine is not an artifact produced by the conditions of this experiment, it may be that only a relatively small percentage of thyroxine is converted to triiodothyronine. However, since triiodothyronine, once formed, may be further degraded very rapidly, and hence not be recovered from tissue as such, it is not possible to estimate accurately the amount converted.

If such a conversion does actually occur, the mechanism might be simply expressed as follows:



It is probable that rate constant K_2 is at least 3 or 4 times K_1. If this is the case one would expect to find relatively small amounts of triiodothyronine in the tissues involved at any one time, no matter what percentage of thyroxine is degraded via this pathway.

It may be argued that only small amounts of triiodothyronine were recovered and this only relatively infrequently because of poor recovery during the experimental procedure. This point may be answered in part by pointing out that when stock triiodothyronine was subjected to the experimental chromatographic conditions, more than 95% was recovered as triiodothyronine. However, under our experimental conditions we have consistently observed a "front" peak of radioactivity which is recovered from the kieselguhr columns before the thyroxine peak appears. (Figs. 1, 2, 3). Efforts to identify the radioactive components of this peak by paper chromatographic techniques have so far been unrewarding and it is entirely possible that small amounts of triiodothyronine may be included in this material. This might explain the appearance of triiodothyronine, following the administration of thyroxine, at some times and not at others.

Similarly, it may be argued that the appearance of triiodothyronine after administration of thyroxine was due to deiodination produced by the conditions of the experiment rather than to tissue activity. However, when thyroxine stock solution was subjected to the experimental conditions no triiodothyronine was recovered.

The appearance of triiodothyronine in the subcellular fractions in greater concentrations than in the whole homogenate is as yet unexplained. The largest amounts appeared in the microsomes and residual fraction in both liver and kidney, probably due to concentration in these fractions. Concentration alone, however, does not explain the consistent appearance of triiodothyronine in the subcellular fractions in contrast to its irregular appearance in the whole homogenate, since the concentration in all cell fractions was higher than that observed in the whole homogenate. It was noted during the course of our experiments that the "front" peak was consistently smaller in the instances where the subcellular fractions were subjected to kieselguhr chromatographic separation than was the case with the whole homogenate, probably due to minor alterations in the experimental conditions. If this "front" contains appreciable amounts of triiodothyronine this difference might explain the more constant appearance of this substance in the analyses where the "front" was smaller.

Our observations have indicated that a small amount of thyroxine may be converted to triiodothyronine in rat tissues. However, it seems less likely that the main mode of action of thyroxine involves this step. In view of the recent works of Thibault and Pitt-Rivers(20), the possibility seems stronger that both substances exert their action only after further alteration, perhaps involving conversion to the acetic acid or other desamino analogues.

Summary. (1) I^{131}-labeled thyroxine was administered intravenously to normal and thyroidectomized rats and the animals sacrificed at varying time intervals thereafter. Specimens of liver, kidney and muscle were homogenized, extracted with butanol and the extracts were subjected to kieselguhr chromatographic analysis. Small amounts of triiodothyronine were recovered from all 3 tissues in about 20% of the analyses carried out. (2) When triiodothyronine was administered and the tissues subjected to the same conditions, no thyroxine was recovered. (3) When

the tissues of rats given thyroxine were homogenized and subjected to fractionation into their subcellular components, triiodothyronine was more readily recovered than was the case where the whole homogenate was used. A possible explanation for this is discussed.

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Intracellular Potassium Concentration in Normal and Diabetic Rat Liver.* (21950)

LOUIS TOBIAN,† WILLIAM I. MORSE,‡ AND A. BAIRD HASTINGS.
From the Department of Biological Chemistry, Harvard Medical School, Boston, Mass.

It has previously been shown that marked abnormalities of carbohydrate metabolism occur in liver slices from normal animals incubated under conditions leading to lowered intracellular potassium concentration(1). Thus, potassium depletion leads to decreased glycogen deposition from glucose and to decreased overall glucose utilization, while gluconeogenesis from pyruvate and other precursors is markedly increased. These metabolic abnormalities are qualitatively similar to those found in liver slices from diabetic rats even in the presence of normal intracellular potassium concentration(2,3). Furthermore, a number of observations have been made, both clinically and in experimental animals, suggesting that potassium depletion may lead to disturbances of carbohydrate metabolism qualitatively similar to those of diabetes mellitus(4,5). It seemed of interest, therefore, to determine whether lowered intracellular potassium concentration occurred in diabetic liver *in vivo*. Accordingly, electrolyte, water and glycogen analyses were made on diabetic and normal rat livers.

Material and methods. Male rats weighing 250 g were made diabetic by the intravenous injection of alloxan (40 mg per kilo). Three to 4 weeks later these animals, as well as normal controls, were fasted for 8 hours (water *ad lib.*), then sacrificed for blood and liver analysis. The animals were sacrificed under nembutal anesthesia by severing the carotid

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† Established Investigator of the American Heart Assn.

‡ R. Samuel McLaughlin Traveling Fellow in Medicine.