The Influence of Fasting, Diabetes, and Several Pharmacological Agents on the Pathways of Thyroxine Metabolism in Rat Liver

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ABSTRACT As judged from both paper and column chromatography, slices or homogenates of liver from rats fasted for 48 h displayed a lesser rate of generation of ^{125}I-labeled 3,5,3′-triiodothyronine (T_3) from ^{125}I-labeled thyroxine (T_4) added to incubation media than did preparations from normal chow-fed animals. A similar defect in the conversion of T_4 to T_3 in the livers of fasted animals was observed when preparations were incubated with substrate concentrations of T_4 so that T_3 generation could be assessed by radioimmunoassay. The effect of fasting could be prevented, wholly or in part, by administration of glucose in the drinking water to otherwise fasted animals, and the degree of prevention appeared to be proportional to the concentration of glucose employed. Diminished generation of T_3 from T_4 was similarly evident in the livers of animals with streptozotocin-induced diabetes mellitus, and this defect was overcome by the provision of insulin in vivo, but not in vitro. Decreased formation of T_3 from T_4 was also observed in preparations of liver from animals given dexamethasone, amiodarone, and propylthiouracil. In no case could these effects on the net formation of T_3 from T_4 be explained by effects of the experimental conditions on the degradation of the T_3 generated, as judged from the rate of degradation of exogenous ^{125}I-T_3 measured in parallel incubates.

An analysis of the rate of disappearance of ^{125}I-T_4 from reaction mixtures in relation to the rate of appearance of ^{125}I-T_3 and ^{125}I-iodide was employed to estimate the activity of the 5-monodeiodinating pathway of T_4 metabolism that leads to the formation of 3,3′,5′-triiodothyronine (reverse T_3). Such estimates indicated that reverse T_3 formation was actively proceeding in the preparations studied, was slightly enhanced by fasting, was unaffected by dexamethasone and amiodarone, and was markedly inhibited by propylthiouracil.

In view of the similarities between the effect of these experimental manipulations on the generation of T_3 from T_4 by rat liver in vitro to their effects on the production rates and serum concentrations of T_3 in man, it is concluded that the rat liver system provides a suitable model for the study of factors that influence the conversion of T_4 to T_3 in man. In addition, the findings strongly indicate that this process, at least in the liver, is closely linked to the utilization of carbohydrate.

INTRODUCTION

Recent studies have provided convincing evidence that 3,5,3′-triiodothyronine (T_3)[1] contributes a major portion to the overall metabolic action of the thyroid hormones within the peripheral tissues. It is evident, moreover, that much of the T_3 found in the blood of normal man arises, not by direct secretion from the thyroid gland, but rather from the peripheral monodeiodination of thyroxine (T_4) in its outer ring at the 5′-position. It is generally agreed that this reaction results in activation of the hormone molecule, as T_3 is several times more potent than T_4 in most all metabolic respects. An alternate pathway of peripheral T_4 metabolism involves monodeiodination of T_4 in its inner ring to yield 3,3′,5′-triiodothyronine (reverse T_3, rT_3), a product that appears to be hormonally inactive, or nearly so. This pathway is, therefore, considered to be one leading to hormonal inactivation. Evidence in support of these conclusions has been extensively discussed in recent reviews (1, 2).

If the foregoing formulation is correct, then any situa-

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[1] *Abbreviations used in this paper*: PTU, propylthiouracil; T_3,3,5,3′-triiodothyronine; T_4, thyroxine; rT_3, reverse T_3.

tion associated with an alteration in the relative activity of these two pathways should result in altered availability to the tissues of active thyroid hormone. As judged from concentrations of T_3 and rT_3 in serum, such shifts do indeed occur. A diversity of conditions has been shown to be associated with decreases in serum T_3 concentration, among them starvation (3–6), acute or severe chronic illness (7, 8), operative stress (9, 10), anorexia nervosa (11–13), and the administration of glucocorticoids (14–16), the adrenergic blocking agents amiodarone (17), or propranolol (18), or propylthiouracil (PTU) (19, 20). In many of these situations there occurs an accompanying increase in serum rT_3 concentration (4, 6, 8, 15, 17). In the case of patients with hepatic cirrhosis (21–23) or those undergoing starvation (24), decreased peripheral generation of T_3 from T_4 has been directly demonstrated. It would appear, therefore, that the pathway leading peripherally to the generation of T_3 from T_4 may be an important metabolic control point with respect to thyroid hormone action, one whose activity is subject to modification by factors as yet unknown.

In an effort to clarify the mechanisms that influence, and perhaps control, the processes by which T_3 is generated from T_4 in man, we have sought an in vitro model using animal tissue that would closely reflect the influence of factors known to or believed to affect the production of T_3 from T_4 in man. Preparations of rat liver have been shown in the present studies to fulfill this criterion, in that they displayed decreased generation of T_3 from T_4 when obtained from animals subjected to starvation, the diabetic state, or treatment with dexamethasone, amiodarone, or PTU. These and other influences on hepatic T_3-neogenesis form the subject of this report. A portion of these findings has been presented in abstract form (25).

METHODS

Isotopes, chemicals, and diet. All hormones, whether isotopically labeled or unlabeled, as well as chemicals, drugs, and laboratory chow, were obtained from commercial sources[2].

Animals. Experiments were performed with the use of tissues obtained from Sprague-Dawley rats of the CD strain (Charles River Breeding Laboratories, Wilmington, Mass.). Although the weights of the animals used in the various experiments varied between 150 and 250 g, the rats were closely matched for weight within a single experiment. Unless otherwise stated, animals were maintained on an ad lib. regimen of tap water and a standard pelleted laboratory chow, comprising 14% protein, 6% fat, and 54% carbohydrate for 1 wk before and during experiments.

Fasting and glucose replacement. In these experiments, a group of the animals was totally deprived of laboratory chow (fasted animals) for 48 h before sacrifice whereas fed controls were allowed continued access to food. In some experiments, some of the fasted animals were given solutions of glucose in place of drinking water, starting at the time that food was withdrawn.

Experimental diabetes. Diabetes mellitus was induced by the administration of a single intravenous dose of streptozotocin, 6.5 mg/100 g body wt. 1 day later, all animals so treated developed hyperglycemia and glycosuria without ketonuria, which persisted, in the absence of treatment, until the end of the experiment. Some of the diabetic animals were given a subcutaneous dose of protamine zinc insulin (3 U/100 g body wt) daily on the 2nd, 3rd, and 4th days of the experiment. This dose resulted in correction or amelioration of glycosuria and polyuria. Both untreated and treated diabetic animals, as well as controls, were allowed free access to laboratory chow until they were killed on the 5th day of the experiment.

Laparotomy. Under light ether anesthesia, wide vertical and horizontal incisions were made extending into the peritoneal cavity of the rat; muscle layers were then apposed with sutures and skin flaps were stapled together. After the procedure, which lasted approximately 20 min, animals were provided chow, fasted, or supplied glucose water only for the following 48 h.

Drug treatments. In some experiments, animals were treated with dexamethasone, amiodarone, or PTU according to treatment schedules described in the appropriate portion of the results section.

Preparation and incubation of slices. At the termination of each treatment regimen, animals were killed by cervical subluxation and the liver was quickly excised. With the aid of a Stadie-Riggs microtome, liver slices of uniform thickness, weighing ~200 mg, were prepared. After an initial weighing, slices from control and experimental animals were closely matched in weight by trimming. They were then suspended in vials containing 2 ml of Krebs-Ringer phosphate buffer, pH 7.4, enriched with either ^{125}I-T_4 (1 μCi/ml; 0.020 μg/ml) or ^{125}I-T_3 (1.3 μCi/ml; 0.025 μg/ml). In experiments in which T_3 generation was to be assessed by radioimmunoassay, media were enriched with stable T_4 (5 μg/ml). Vials were either incubated under room air or were closed by rubber stoppers and gassed continuously with purified N_2. Incubations were carried out at 37°C for 4 h. In most experiments, some vessels were incubated at 37°C, but without tissues, and others containing tissue slices were incubated at 0°C. No generation of ^{125}I-T_3 from ^{125}I-T_4 was observed in these controls, indicating the absence of artifactual conversion of T_4 to T_3 either during incubation or during chromatography.

At the end of incubation, vessels were plunged into cracked ice, and, when cold, the slices were homogenized in their own medium. Homogenates were then mixed with outdated blood bank plasma to inhibit any further metabolism of T_4, and the mixture was frozen and kept for 1 wk at most until paper chromatographic analysis was performed.

Preparation and incubation of homogenates. Pieces of liver were homogenized in Krebs-Ringer phosphate (1:3,

[2] ^{125}I-T_4 (sp act = 50–75 μCi/μg) and ^{125}I-T_3 (sp act = 50–75 μCi/μg) were purchased from Abbott Diagnostics, Diagnostic Products (North Chicago, Ill.) ^{131}I-T_3 (sp act = 85 μCi/μg) was purchased from Industrial Nuclear Corporation (St. Louis, Mo.). Stable T_4, T_3, and PTU were purchased from Sigma Chemical Co. (St. Louis, Mo.). NPH Iletin and regular insulin from Eli Lilly and Company (Indianapolis, Ind.). Streptozotocin was obtained from The Upjohn Company (Kalamazoo, Mich.), dexamethasone sodium phosphate (Decadron phosphate) from Merck, Sharp & Dohme (West Point, Pa.,), and amiodarone [2-butyl-3-(4'-diethylaminoethoxy-3',5'-diiodobenzoyl) benzofurane] from La Bax, Brussels, Belgium. Pelleted laboratory chow, RMH 1000, was purchased from Agway-Country Foods, Agway Inc. (Syracuse, N. Y.).

wt/vol). Homogenates were enriched with ^{125}I-T_4 (1 μCi/ml) or ^{125}I-T_3 (1.3 μCi/ml) and incubated under room air or N_2 for 4 h, as described above for tissue slices, previous experiments having shown that generation of T_3 from T_4 increased in a linear manner during the first 6 h of incubation. In most experiments, control vessels containing homogenates were comparably incubated at 0°C. At the end of incubation, homogenates were mixed with outdated blood bank plasma (1:2) and the mixtures were frozen until chromatographic analysis was performed.

Paper chromatography. Homogenates were thawed and mixed vigorously, and 10-μl aliquots were applied to Whatman 3 MM filter paper strips together with carrier iodide, T_4, T_3, and, in some instances, rT_3 or tetraiodothyroacetic acid. Chromatograms were developed in a descending hexane, tertiary amyl alcohol, 2 N ammonia (1:10:11) solvent system.[3] After development and drying of the chromatographic strips, iodothyronines were localized by fluorescent light, and iodide by staining with 0.1% palladium chloride. Zones corresponding to carriers, as well as to the origin, were excised and counted in a well-type scintillation counter. Preliminary experiments revealed that >98% of the ^{125}I in the entire strip was present in these zones. Radioactivity in a given zone was calculated as a percent of the total ^{125}I in these areas. Values for the percentage generation of the several products of T_4 metabolism were always corrected for the percentage contamination by each in the substrates employed as assessed by paper chromatography. In the case of substrate ^{125}I-T_4, 96% of the total radioactivity was T_4, between 0.2 and 0.5% was T_3, and ~2% was ^{125}I-iodide. Substrate ^{125}I-T_3 was at least 95% pure and contained from 0.5 to 0.8% ^{125}I-iodide.

Sephadex chromatography. In a number of instances, the generation of ^{125}I-T_3 from ^{125}I-T_4 in liver slices incubated in air was assessed by chromatography on columns of Sephadex G-25 superfine in a modification (26) of the method described by Green (27). Incubation mixtures comprising slices homogenized in their media were diluted with human plasma (1:9, vol/vol) containing 8-anilino-1-naphthalene sulfonic acid (9 mg/ml) and marker ^{131}I-T_3. The resulting mixture was applied to a 25 × 2-cm column containing preswollen Sephadex. The ^{125}I-T_3 peak was clearly separated from substrate ^{125}I-T_4. The T_3 generated was measured as the fractional contribution of the counts in the T_3 peak to the total counts eluted.

Stable T_3 and T_4 assays. The concentrations of T_3 and T_4 in rat plasma were measured directly by radioimmunoassay and competitive binding techniques, respectively. In some experiments, the generation of stable T_3 from T_4 in liver slices was measured using a radioimmunoassay technique to assess the quantity of T_3 formed. After incubation in air, slices were homogenized in their media and then extracted with 5 vol of 95% ethanol. In separate studies with ^{125}I-T_3, this was shown to extract >95% of the T_3 present. 10 or 20 μl of extract was rapidly evaporated under nitrogen, the T_3 was solubilized in rat serum freed of T_3 by charcoal adsorption, and the T_3 content was assayed. Preliminary studies indicated that 10–20 μl of tissue extract incubated without substrate T_4 could be introduced into the radioimmunoassay without influencing the configuration of the standard curve. Negligible generation of T_3 from T_4 was detected in simultaneously incubated tissue-free blanks.

Statistical evaluation of data. In experiments with two groups of animals, statistical analysis was performed using the Student's *t* test. When three or more treatment groups were studied simultaneously, a two-way analysis of variance was performed. Duncan's Multiple Range Test was then applied to identify significant differences among specific groups (28).

RESULTS

The major purpose of these studies was to examine the effects of a variety of experimental manipulations on the generation of T_3 from T_4 in rat liver. In each experiment with ^{125}I-T_4, however, the disappearance of labeled T_4, and generation of labeled iodide, origin material, tetraiodothyroacetic acid, rT_3, and conjugates of the iodothyronines was also examined. Detectable quantities of tetraiodothyroacetic acid, rT_3, and conjugates were not evident, only labeled T_3, iodide, and origin material being seen. The generation of labeled origin material ranged from 1 to 6% of added ^{125}I-T_4, was not materially altered by any experimental manipulation, and will not be described further.[4] For purposes of brevity, results with respect to the generation of iodide will not be discussed, except when significant changes in the generation of this product were seen.

Effects of fasting and glucose feeding. Generation of ^{125}I-T_3 from ^{125}I-T_4 was markedly reduced in slices of liver from fasted animals, whether incubated in air or in nitrogen (Fig. 1). This effect was completely prevented by providing 25% glucose in place of drinking water to animals whose food had been withdrawn. Indeed, in some experiments, the rate of T_3-neogenesis was greater in livers from glucose-fed than from chow-fed animals, but this difference was not statistically significant. In liver slices, generation of ^{125}I-T_3 was slightly, but not significantly, greater when incubations were conducted under nitrogen than when room air was employed.

Studies in slices from the same animals revealed that the changes in apparent ^{125}I-T_3 generation induced by fasting and by glucose replacement could not be explained by differences in the rate of deiodination of T_3, as judged from the disappearance of added ^{125}I-T_3, since T_3 disappearance was either the same or less rapid in slices from fasted animals than in those from fed controls (Fig. 1).

Entirely comparable results with respect to the effects of fasting and glucose replacement were observed in homogenates of rat liver, regardless of whether incubations were conducted in air or under nitrogen (Fig. 2). Here, however, as previous workers have found in rat kidney (29), a much greater generation of ^{125}I-T_3 under anaerobic rather than under aerobic conditions was observed. Again, differences

[3] The *Rf* values for carrier and(or) labeled compounds were: I^-, 0.11; rT_3, 0.23; T_4, 0.30; 3,3'-diiodothyronine (3,3'-T_2), 0.38; tetraiodothyroacetic acid, 0.43; T_3, 0.56.

[4] For the experiments shown in Table I, the percentage generation of ^{125}I-labeled origin material can be calculated from the formula [A-(B + C)].

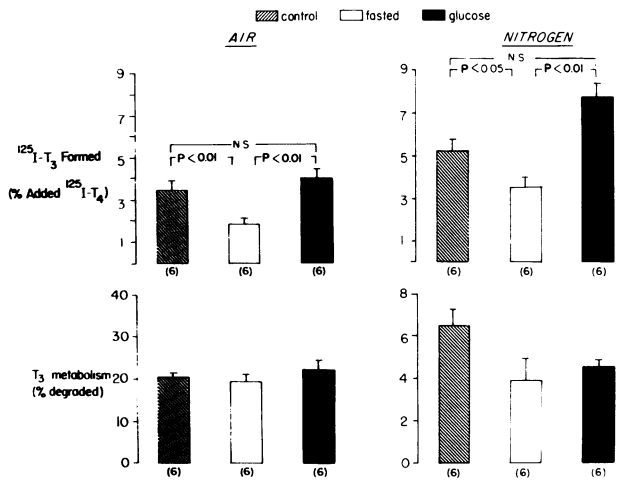

FIGURE 1 Effect of fasting for 48 h or the provision of a 25% solution of glucose in the drinking water to food-deprived animals on the formation of ^{125}I-T_3 from ^{125}I-T_4 in rat liver slices. T_3 metabolism was assessed in separate slices from the same animals incubated with ^{125}I-T_3 in air or nitrogen. In this and subsequent figures, bars and vertical brackets depict the mean and standard error of values obtained in the number of animals shown in parentheses, and absence of an indicated *P* value denotes lack of statistical significance between groups.

among the three experimental groups in respect to the rate of degradation of added ^{125}I-T_3 could not explain the differences that were observed in the generation of ^{125}I-T_3 from ^{125}I-T_4.

In the case of homogenates incubated under nitrogen, fasting was associated with significant decreases of about 50% both in the disappearance of ^{125}I-T_4 from the reaction mixture and in the generation of ^{125}I-labeled iodide.

The ability of glucose replacement to prevent the reduction in T_3 generation seen in livers from fasted animals was related to the concentration of glucose provided in the drinking water. In studies of liver slices incubated in air, in vivo administration of 1% glucose had no effect, whereas both 5 and 25% glucose significantly increased T_3 generation to values higher than those seen in slices from fasted animals. Moreover, generation of T_3 from T_4 was greater when 25%, rather than 5%, glucose was administered (Fig. 3).

In contrast to the effects of glucose administration in vivo, enrichment of suspending media with glucose (5 mM), with or without insulin (0.001–1 μM), had no effect on the generation of T_3 from T_4 by liver slices from fasting animals (data not shown).

To validate the data obtained by paper chromatography of specimens incubated with ^{125}I-T_4, two groups of experiments were performed. In the first, ^{125}I-T_3 generation by rat liver slices was assessed by chromatography of reaction mixtures on Sephadex columns, and the values obtained were compared to those obtained by paper chromatography. Close agreement between the two methods of analysis was noted, and fasting was again associated with decreased generation of T_3 (Fig. 4).

In the second group of experiments, radioimmunoassay was employed in place of isotopic assay to assess the effects of fasting and glucose replacement on T_3 generation by rat liver slices incubated in

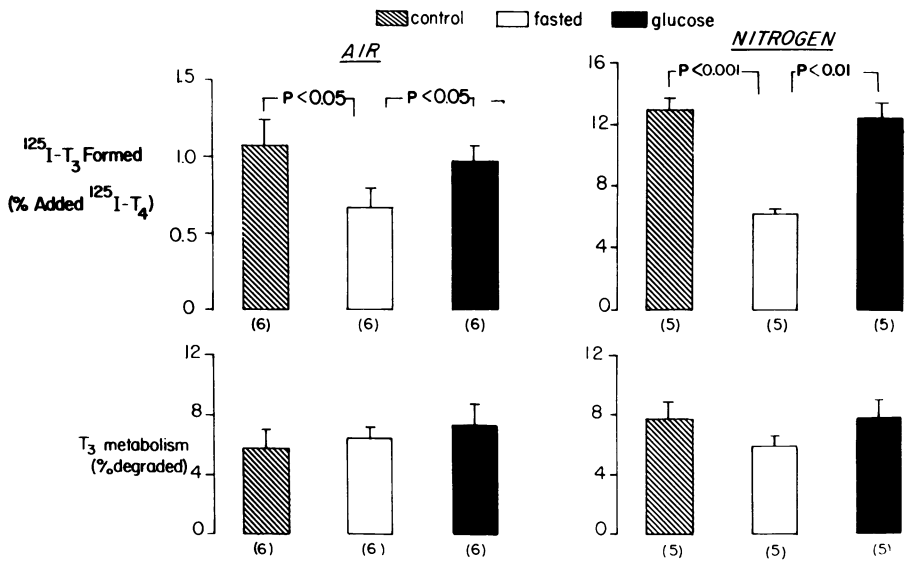

FIGURE 2 Effect of fasting or provision of a 25% solution of glucose in the drinking water for 48 h on the formation of ^{125}I-T_3 from ^{125}I-T_4 in homogenates of rat liver incubated in air or nitrogen. T_3 metabolism was assessed in parallel incubations with ^{125}I-T_3.

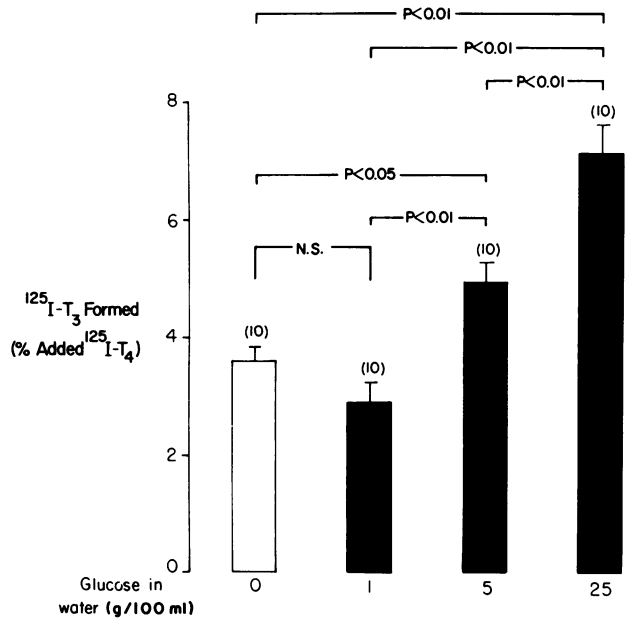

FIGURE 3 Generation of ^{125}I-T_3 from ^{125}I-T_4 by liver slices of fasted animals maintained on varying concentrations of glucose in the drinking water for 48 h. Incubations were performed in room air. Numbers below the bars indicate the concentration of glucose in the drinking water.

air (Fig. 5). The results obtained were completely confirmatory of those obtained by the isotopic technique.

Effect of thyroid hormone replacement on the response to fasting. Since it is known that thyroid function decreases in the fasted rat, studies were undertaken to determine what role, if any, hypothyroidism might play in the decreased generation of T_3 from T_4 seen in the livers of animals fasted for 48 h. In confirmation of previous findings, a 48-h fast was associated with a highly significant decrease ($P < 0.001$), both in serum T_4 concentration (control, 4.3±0.2; fasted, 1.4±0.2 μg/dl) (mean±SE) and in serum T_3 concentration (control, 39.6±1.4; fasted, 10.8±1.4 ng/dl).

Despite these changes, administration of two daily doses of either T_4 (1.5 μg/100 g body wt) or T_3 (0.5 μg/100 g body wt) during the 48-h period of fasting failed to influence the reduction in ^{125}I-T_3 generation from ^{125}I-T_4 seen in liver slices of matched animals not given exogenous hormone (data not shown).

Effect of experimental diabetes. Rats given streptozotocin and allowed free access to food became manifestly diabetic 2 days later and remained so during the ensuing 3 days. As compared to findings obtained in controls at that time, liver slices obtained from diabetic rats displayed greatly reduced generation of ^{125}I-T_3 from ^{125}I-T_4 (Fig. 6). This effect was almost completely reversed by the administration of protamine zinc insulin (3 U/100 g body wt) daily during the last 3 days of the experiment. However, no reversal of the impairment in T_3 generation was observed when liver slices from diabetic rats were incubated in media containing glucose (5 mM) and insulin (1μM).

The effects of diabetes and of insulin replacement on the apparent generation of ^{125}I-T_3 from ^{125}I-T_4 could not be explained by an alteration in the rate of T_3 degradation, as judged from the disappearance of added ^{125}I-T_3 in vessels containing matched slices (Fig. 6). Untreated diabetes was associated with a small, but not statistically significant, decrease in the disappearance of ^{125}I-T_3 from the reaction mixture. Moreover, when T_3 degradation by liver slices from diabetic animals treated with insulin in vivo was compared to that of liver slices from diabetic animals incubated with insulin in vitro, a significantly ($P < 0.05$) slower T_3 disappearance was evident in the latter group.

Effect of laparotomy. Groups of five animals were fasted, provided laboratory chow, or supplied glucose in the drinking water for 48 h after laparotomy, whereas unoperated controls were maintained on parallel regimens. Mean values for the percent generation of T_3 from T_4 in liver slices from control and operated animals, respectively, were: chow-fed, 4.3±0.3 vs. 4.8±0.7; fasted, 2.3±0.3 vs 1.9±0.2; glucose-fed, 4.6 ±0.3 vs. 4.7±0.6. Analysis of variance confirmed to the characteristic effects of the dietary regimens, but demonstrated no effect of laparotomy.

Effects of various drugs on T_3 generation from T_4 (Table I). An approximate 50% inhibition of T_3 generation from T_4 was observed in liver slices from animals given 1.0 mg of dexamethasone subcutaneously daily for 3 days before sacrifice. However, no effect on ^{125}I-T_4 disappearance or generation of labeled iodide was observed.

Profoundly inhibited generation of ^{125}I-T_3 from ^{125}I-T_4 was seen in liver slices from chow-fed animals given amiodarone as a 0.1% solution in 25% glucose for 3 days in place of the drinking water. Amiodarone also produced a slight, but statistically insignificant, decrease in ^{125}I-T_4 disappearance and a slight, but significant, decrease in the generation of labeled iodide.

PTU, when given in the drinking water as a 0.05% solution in 25% glucose for 3 days, almost completely abolished the generation of ^{125}I-T_3 from ^{125}I-T_4 by liver slices. Concomitantly, disappearance of ^{125}I-T_4 and generation of ^{125}I-labeled iodide were markedly inhibited.

Effect of various drugs on the metabolism of T_3 (Table II). Animals treated with dexamethasone and PTU displayed slight, statistically insignificant reductions in the rate of disappearance of T_3, whereas those given amiodarone showed no change. Significantly decreased generation of ^{125}I-iodide from added ^{125}I-T_3 was observed after treatment with all three agents.

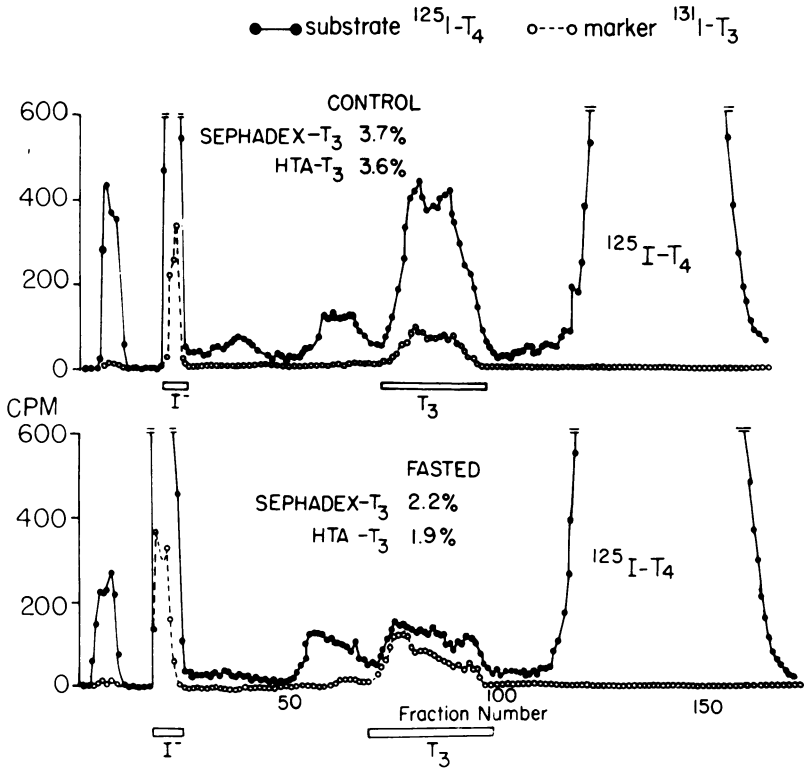

FIGURE 4 Assessment of ^{125}I-T_3 generation from ^{125}I-T_4 by column chromatography of reaction mixtures on Sephadex G-25 superfine. Liver slices from rats fasted for 48 h or from fed controls were incubated with ^{125}I-T_4 and then homogenized in their media and homogenates were subjected to chromatography either on columns of Sephadex or on filter paper using a hexane-tertiary amyl alcohol-ammonia solvent system (HTA). Shown in the figure are elution patterns obtained during column chromatography of specimens from fed or fasted animals. Values for the percent generation of T_3 from T_4 as judged from paper chromatography (HTA-T_3) are compared to those obtained from column chromatography (Sephadex-T_3).

DISCUSSION

In the present studies, we have sought to determine whether the rat liver constitutes a reasonable animal tissue model in which to study the factors that influence the peripheral conversion of T_4 to T_3 in man.

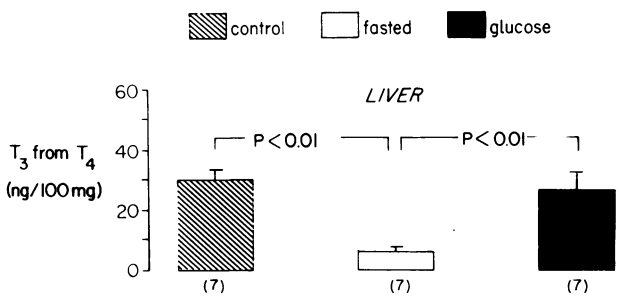

FIGURE 5 Effect of fasting or provision of a solution of 25% glucose in the drinking water on the generation of T_3 from T_4 in liver slices, as judged from radioimmunoassay. Incubations were performed in air with T_4, 5 μg/ml, added to the medium.

For this purpose we have determined the effect in this tissue of a variety of experimental manipulations that have been shown to inhibit the conversion of T_4 to T_3 in man, as judged from changes in serum T_3 concentration, T_3 production rate, or both. The data suggest that rat liver slices or homogenates are valid models, since excellent concordance was observed between changes in T_3 generation from T_4 by rat liver preparations and alterations in T_3 metabolism in man in response to starvation or to the administration of dexamethasone, amiodarone, and PTU.

The apparent inhibitory effect of these experimental manipulations on T_3 generation from T_4 cannot be explained by an effect on the degradation of the T_3 generated, at least as judged from the metabolism of exogenous T_3 within these systems. Degradation of exogenous T_3 was slow, and was retarded, rather than accelerated, by the factors that decreased T_3 generation.

In previous studies of the generation of T_3 from T_4 by animal tissues in vitro, either paper chromatog-

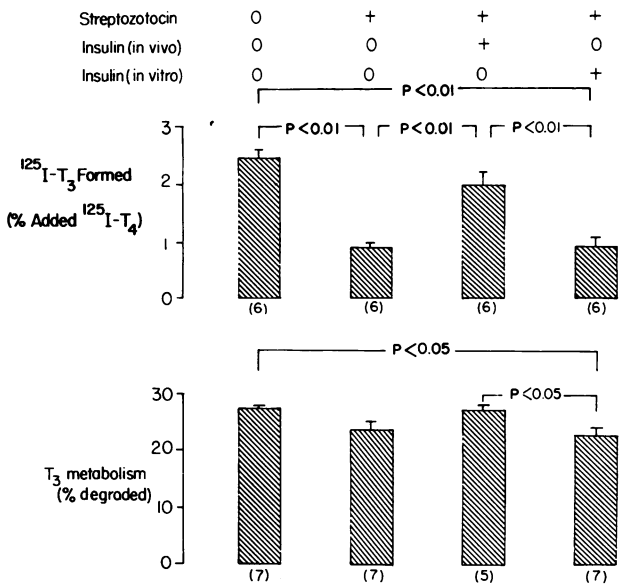

FIGURE 6 The effect of streptozotocin-induced diabetes mellitus and of insulin replacement on the generation of ^{125}I-T_3 from ^{125}I-T_4. The formation of ^{125}I-T_3 from ^{125}I-T_4 and the metabolism of ^{125}I-T_3 were assessed in parallel incubations of liver slices in air. Diabetic animals either received injections of saline or protamine zinc insulin, 3 U/100 g for 3 days. Slices from diabetic animals were incubated in unsupplemented medium or in media containing 1 μM insulin, and 5 mM glucose.

raphic analysis of preparations incubated with radioiodine-labeled T_4 or radioimmunoassay of preparations enriched with unlabeled T_4 has been used as a means of assessing T_3 generation (29–34). In the present studies, we have mainly employed the isotopic-paper chromatographic technique, and have validated the results obtained both by column chromatography of isotopically labeled preparations and by radioimmunoassay.

Few specific conclusions can be drawn as to the mechanisms by which the various experimental manipulations inhibit T_3 generation from T_4. The similar effects of starvation and streptozotocin diabetes are not surprising, perhaps, in view of the variety of metabolic alterations common to both, including insulinopenia, and diminished activity of a variety of enzymes associated with glucose metabolism (35, 36). Similarly, the ability of glucose administration to prevent the effect of fasting, and of insulin in vivo to reverse the effect of experimental diabetes, suggests that, in the liver at least, the metabolism of glucose is somehow related to the process of T_3 formation. A relationship between glucose metabolism and the generation of a cofactor necessary for T_3 formation is suggested by our finding that the T_3-generating activity of broken cell systems from the livers of fasted rats is restored to normal by enrichment with an NADPH-generating system (isocitrate; isocitrate dehydrogenase; NADP, 0.1 mM).[5]

The question arises as to whether T_3-neogenesis is specifically linked to carbohydrate utilization or whether it is more generally related to the caloric adequacy of the diet. Evidence that it is not solely a function of caloric intake is provided by studies conducted in our laboratory (data not reported) which

[5] Balsam, A., and S. H. Ingbar. Unpublished observations.

TABLE I
*The Metabolism of ^{125}I-T_4 in Slices of Rat Liver**

Treatment	Treatment group	Number of animals	Measured values: (A) ^{125}I-T_4 degradation (% added T_4)	Measured values: (B) ^{125}I-T_3 generation (% added T_4)	Measured values: (C) ^{125}I-Iodide generation (% added T_4)	Derived values: Excess ^{125}I-I^- (% added T_4) (C − B)	Derived values: Excess I^-/T_4 degradation (C − B)/A	Derived values: T_3 generation/T_4 degradation (2B/A)	Derived values: Excess I^-/^{125}I-T_3 generation (C − B/B)
Fasting	Control	18	18.4±1.3	3.0±0.3	10.3±0.9	7.3±0.7	0.40±0.02	0.32±0.02	2.8±0.3
	Exp.	18	18.0±1.2	1.6±0.2‖	10.1±0.8	8.5±0.7‡	0.47±0.02‖	0.17±0.01‖	5.7±0.4‖
Diabetes	Control	4	30.8±2.0	6.5±0.5	23.8±2.1	17.3±2.2	0.56±0.05	0.43±0.04	2.7±0.5
	Exp.	4	24.5±1.6	3.7±0.4§	19.2±2.1	15.5±2.4	0.62±0.05	0.31±0.04	4.6±1.3
Dexamethasone	Control	6	36.5±3.4	5.1±0.5	25.0±2.3	19.9±2.6	0.54±0.05	0.30±0.05	4.2±0.8
	Exp.	6	36.4±3.4	2.7±0.4‡	24.1±2.7	21.4±2.5	0.62±0.03‡	0.16±0.02‡	8.4±1.0‡
Amiodarone	Control	5	34.9±2.8	6.3±0.8	26.9±1.9	20.6±1.4	0.60±0.03	0.36±0.03	3.5±0.5
	Exp.	5	26.1±1.6	0.5±0.02§	21.8±1.2‡	21.2±1.3	0.82±0.01§	0.04±0.004‖	42.2±3.8‖
PTU	Control	5	29.7±1.4	7.0±0.08	17.8±0.9	10.8±0.5	0.37±0.02	0.47±0.04	1.7±0.3
	Exp.	5	14.0±0.6‖	0.6±0.04	6.6±0.7‖	6.0±0.7§	0.42±0.03	0.09±0.002‖	9.8±0.6‖

* Data shown represent mean±SE. See text for precise description of experimental manipulations.
‡ $P < 0.05$.
§ $P < 0.01$.
‖ $P < 0.001$.

TABLE II

*The Metabolism of ^{125}I-T_3 in Slices of Rat Liver**

Treatment	Treatment group	No. of animals	T_3 degradation (% added T_3)	Iodide generation (% added T_3)
Fasting	Control	6	20.6±0.8	16.9±1.0
	Exp.	6	19.4±1.8	13.5±1.5
Diabetes	Control	7	27.3±1.0	20.1±0.6
	Exp.	7	23.7±1.3‡	16.2±1.2§
Dexamethasone	Control	4	19.8±1.7	17.2±1.8
	Exp.	5	16.5±2.8	11.0±1.9‡
Amiodarone	Control	6	20.4±2.6	13.7±1.5
	Exp.	6	19.5±1.9	7.2±0.9§
PTU	Control	6	20.4±2.6	13.7±1.5
	Exp.	6	16.3±0.4	5.5±0.5‖

* Data shown represent mean±SE. See text for precise description of experimental manipulations.
‡ $P < 0.05$.
§ $P < 0.01$.
‖ $P < 0.001$.

demonstrate that provision of fat in the form of an emulsion of peanut oil in the drinking water (5% vol/vol), which the animals readily consumed, failed to influence the defective formation of T_3 observed in livers of animals deprived of chow. In fasting man, moreover, carbohydrate diets are more effective than equicaloric quantities of protein in restoring depressed serum T_3 concentrations to normal (6), and the lesser effect of protein can itself perhaps be ascribed to enhancement of gluconeogenesis.

In man, a decrease in serum T_3 concentration occurs in association with a variety of "stressor" stimuli, such as surgery (9, 10), and acute and chronic illnesses of wide variety of types (7, 8). Nonspecific stress does not appear to be a factor in the present in vitro system, however, since wide laparotomy failed to influence the hepatic generation of T_3 from T_4. This finding also makes it unlikely that the effect of starvation or diabetes can be ascribed to adrenocortical activation, a possibility suggested by the inhibition of hepatic T_3 generation induced by dexamethasone, albeit in very large doses, that we and others (32) have found.

Considerable evidence indicates that the peripheral metabolism of T_4 in man proceeds mainly by a sequence of monodeiodinations occurring randomly in locus, though not in rate, at the inner and outer rings of the thyronine nucleus (37). Although it has obviously been possible to assess the rate of T_3 generation from T_4, as for example in the present and previous studies (29–34), it has generally not been possible to demonstrate generation of rT_3 from T_4 in vitro. Such was certainly the case in the present studies in which ^{125}I-rT_3 was looked for, but never detected, in chromatographic analyses of the reaction mixtures containing labeled T_4. This is probably the result of the extreme rapidity with which rT_3 is itself degraded in tissue systems (38). This being the case, we have attempted to utilize the ancillary data that we have obtained to assess the relative activities of the T_3- and rT_3-generating mechanisms and the manner in which they were affected by the experimental manipulations that were studied. In so doing, we have made the limiting assumption that deiodination of labeled T_3 generated from labeled T_4 is negligible and hence does not influence significantly either apparent T_3 generation or the formation of labeled iodide. If that assumption is made, then estimates of the activity of 5-monodeiodinating pathway, both absolute and relative to that of the 5′-monodeiodinating pathway, can be made.

Since neither conjugates nor deaminated derivatives of T_4 were observed, then the fractional disappearance of ^{125}I-T_4 from the reaction mixtures must have represented the total proportion of added T_4 converted to T_3 and rT_3. Under the assumption that T_3 formed undergoes no deiodination, all ^{125}I-iodide generated in excess of that which could be accounted for by the generation of T_3 must represent ^{125}I-iodide that has passed from T_4 through the rT_3 pathway.[6] Hence, it was possible to obtain an estimate of the activity of this pathway. We have designated this as "excess iodide formation," and have calculated this function as percent ^{125}I-iodide formed minus percent ^{125}I-T_3 generated. This function always comprised a substantial fraction of the T_4 added and indeed always exceeded in magnitude by at least several-fold the measured fraction of T_3 generated (Table I). Activity of the T_3-generating pathway as a fraction of overall T_4 deiodination can be calculated as the ratio, percent T_3 generation/percent T_4 degradation; that of rT_3 pathway as the ratio, percent excess iodide/percent T_4 degradation; the the activities of the two pathways relative to one another as ratio, percent excess ^{125}I-iodide/percent ^{125}I-T_3 generation.

When the current data are examined in this way, several patterns of response to the various experimental manipulations employed emerge (Table I). Fasting decreased T_3 generation, but did not alter significantly either T_4 degradation or iodide generation. Hence, excess iodide generation increased. This indication of a significant, though small, increase in rT_3

[6] It is theoretically possible that a portion of the excess iodide might have arisen from cleavage of the ether linkage of T_4, with deiodination of the outer-ring product of this reaction. Significant activity of any such pathway in rat liver has been excluded, however, by our failure to recover labeled diiodotyrosine when biosynthetically derived, randomly labeled T_4 was incubated with liver slices in the presence of an inhibitor of iodotyrosine deiodinase, dinitrotyrosine (39), in concentrations sufficient to abolish deiodination of added ^{125}I-diiodotyrosine.[5]

production in vitro as a result of starvation, as well as the clear evidence of an increase in the ratio of 5-monodeiodination/5'-monodeiodination, is in accord with the findings of recent studies on the effects of starvation on rT_3 production rates in man (24, 40).

The apparent effect of diabetes differed slightly from that of starvation. T_4 disappearance and total iodide generation were decreased slightly, though not significantly, and no evidence of an absolute increase in 5-monodeiodination was seen, as excess iodide formation was unchanged. Monodeiodination at the 5'-position (T_3 generation) as a function of total T_4 degradation was significantly decreased, whereas 5-monodeiodination, either in relation to total T_4 degradation or to T_3 generation, was increased, though not significantly so.

The effects of dexamethasone and amiodarone were qualitatively similar to those of diabetes in that, although T_3 generation was inhibited, no evidence was obtained that rT_3 formation was increased.

The effects of PTU were strikingly different from those of other experimental manipulations examined. As others have found in studies of T_3 formation in the whole rat (41), PTU given in vivo greatly inhibited T_3-neogenesis in liver slices. In addition, PTU also appeared to inhibit 5-monodeiodination, as judged from estimates of excess iodide formation. The effect on 5-monodeiodination was less marked than the inhibition of T_3 generation, however, since the ratio of excess iodide formation/T_3 generation was greatly increased.

The finding that PTU inhibits the inner-ring pathway of T_4 metabolism is in accord with data recently reported, showing that PTU inhibits the conversion of T_4 to 3,3'-T_2, a metabolite formed largely via the rT_3 pathway in rat liver homogenate (42).

From the above, it emerges that all the manipulations studied resulted in decreased T_3 generation, but that starvation may have enhanced rT_3 formation; diabetes, dexamethasone, and amiodarone did not affect rT_3 formation appreciably, while PTU inhibited rT_3 formation, but less markedly than it inhibited the formation of T_3.

The calculations from which these conclusions are drawn are based upon the assumption that the ^{125}I-T_3 generated from ^{125}I-T_4 does not undergo significant deiodination. It is very unlikely that this is the case, however, since some deiodination of exogenous ^{125}I-T_3 was always observed. Deiodination of endogenously generated T_3 would, by the present methods of calculation, lead to overestimates of "excess iodide formation," a function of the activity of the rT_3 pathway. However, as judged from the only source of information available, i.e., the rate of deiodination of exogenous ^{125}I-T_3, this factor could not have accounted for more than a small proportion of excess iodide formation, since, in relation to the magnitude of these derived values, the proportionate conversion of T_4 to T_3 and the proportionate deiodination of added T_3 were quite small.

For these reasons, the quantitative values of the derived functions must be regarded as approximations only. Nevertheless, we consider them to be, in the main, qualitatively reliable and productive of the only available evidence concerning the activity of the inner-ring monodeiodinating pathway of T_4 metabolism in vitro and the influence thereon of a variety of factors that affect outer-ring monodeiodination.

The findings provide strong evidence that the inner- and outer-ring monodeiodinating pathways for T_4 are biochemically distinct from one another, a conclusion consonant with studies which demonstrate divergent effects of both starvation and chronic liver disease on the production rates of T_3 and rT_3 in man (23, 24).

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