Observations on the Factors that Control the Generation of Triiodothyronine from Thyroxine in Rat Liver and the Nature of the Defect Induced by Fasting

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ABSTRACT Studies were performed to explore the mechanism underlying the impaired generation of ^{125}-I-3,5,3′-triiodothyronine (T_3) from ^{125}I-thyroxine (T_4) (T_3-neogenesis) in preparations of liver from rats fasted for 48 h and the prevention of this effect by the feeding of glucose. T_3-neogenesis in livers from fasted animals and those fed chow or glucose was assessed in various mixtures of crude microsomal fractions with either buffer or cytosols. T_3-neogenesis was mediated by an enzyme present in the microsomal fraction whose activity was enhanced by cytosolic cofactor(s). In livers from animals fasted for 48 h, the supporting activity of cytosol was decreased, whereas the activity of the enzyme was unaffected. Administration of glucose as the sole nutritional source prevented the decrease in the supporting activity of hepatic cytosol that was regularly observed in the case of animals totally deprived of food.

The diminished supporting activity for T_3-neogenesis provided by liver cytosol from fasted animals was restored to normal by enrichment with either NADPH or GSH, but the two cofactors appeared to act at different loci. GSH stimulated T_3-neogenesis in microsomes incubated in the absence of cytosol, i.e., in buffer, whereas NADPH did not. The stimulatory effect of both agents was blocked by the sulfhydryl oxidant, diamide, which also inhibited T_3-neogenesis in mixtures of microsomes with cytosols. Taken together, these observations suggest that GSH acts directly on the enzyme in the crude microsomal fraction, whereas NADPH acts within the cytosol, possibly by increasing the concentration of GSH through the action of the enzyme glutathione reductase, for which NADPH is a cofactor. In this light, the decreased supporting activity of hepatic cytosol from starved animals appears to reflect, at least partly, a decreased concentration of one or both cofactors.

The direct stimulation of enzyme activity by GSH, and the apparent lack of inhibition of unstimulated activity by diamide, suggests that the 5′-monodeiodinase for thyroxine that mediates T_3-neogenesis may be a GSH transhydrogenase.

INTRODUCTION

The demonstration that 3,5,3′-triiodothyronine (T_3)[1] is produced in the peripheral tissues of man by monodeiodination of thyroxine (T_4) in its outer ring has rekindled an intense interest in the deiodinative metabolism of the thyroid hormones and their metabolites (1–4). In man, it has become clear that approximately one-third of the metabolism of T_4 proceeds along a pathway that leads initially to the formation of T_3 (2–4); that in normal individuals peripheral conversion of T_4, rather than thyroid secretion, accounts for most or nearly all of T_3 production (2–4); and that a major portion of the metabolic activity evoked by T_4 may be attributed to its conversion to T_3. It has also become apparent that the peripheral metabolism of T_4 can traverse an alternate pathway, in which the hormone is monodeiodinated in its inner ring to yield 3,3′,5′-T_3 or reverse T_3 (5), a product generally considered to have virtually no hormonal activity (6, 7).

In man, a variety of influences lower the serum T_3 concentration. These include fasting or partial caloric deprivation (8–12); diabetes (13–15); surgical stress (16, 17); moderate or severe chronic illness (18–20); cirrhosis (21, 22); and a number of drugs, including

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[1] *Abbreviations used in this paper*: I^-, iodide; T_3, 3,5,3′-triiodothyronine; T_4, thyroxine; T_3-neogenesis, generation of T_3 from T_4.

propylthiouracil (23, 24), glucocorticoids in high doses (25–27), certain radiographic contrast media (28, 29), and the beta adrenergic blocking agents, propranolol (30) and amiodarone (31). It has generally been assumed that all of these factors act primarily to decrease production of T_3 from T_4, rather than to increase peripheral degradation of T_3, and direct studies have shown this to be true in the case of cirrhosis (5, 22), fasting (12, 32), and diabetes (15).

In previous studies we have demonstrated that the conversion of T_4 to T_3 in rat liver in vitro, an enzymatic process that we have termed T_3-neogenesis, is subject to the influence of the above-mentioned factors known or believed to affect T_3 formation from T_4 in humans (33). Accordingly, we have proposed that T_3-neogenesis in rat liver can serve as a suitable model of this process in man. Our earlier studies revealed that T_3 formation is impaired in preparations of liver from rats fasted for 2 d, and that this effect can be prevented by the administration of glucose. This suggested that T_3-neogenesis is closely linked to glucose utilization, at least in this tissue (33). The present studies were designed to extend previous observations with this model, to further clarify the mechanism of impaired generation of T_3 from T_4 in liver from the fasted rat, and thereby to obtain insight into the factors that regulate this process in normal liver. Portions of this work have been presented in abstract form (34, 35).

METHODS

Hormones and chemicals. Isotopically labeled and stable iodothyronines, as well as the various cofactors and chemical agents employed in these studies, were obtained from commercial sources.[2]

Animals and dietary regimens. Male Sprague-Dawley rats weighing 150–200 g were purchased from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.) and were routinely given a standard diet consisting of pelleted laboratory chow[3] and tap water ad libitum before the study (fed rats). During the final 48-h period, groups of rats were either maintained on the standard diet or deprived of chow but provided free access to drinking water (fasted rats). In other studies, some animals were given the standard diet, whereas others had chow withdrawn but were given free access to a solution of 25% glucose in place of the drinking water (glucose-replaced rats). In experiments involving fasting, all animals were given T_4, 1.5 μg/100 g body weight s.c. daily, as hypothyroidism is known to decrease T_3-neogenesis in rat liver (36, 37), and, in the rat, thyroid hypofunction is a consequence of fasting (33, 38).

Preparation and incubation of microsomes and cytosols from rat liver and kidney. Rats were killed by cervical subluxation, and livers and kidney were rapidly excised and weighed. A portion of liver or the entire kidney was homogenized (2:5, wt:vol) in a sucrose (0.25 M)-phosphate buffer (0.06 M), pH 7.4. The homogenate was centrifuged at 10,000 *g* for 15 min at 4°C, and the pellet was discarded. The supernate was centrifuged at 105,000 *g* for 1 h and the resulting supernate (cytosol) was separated from the sedimented crude microsomal fraction by aspiration. The microsomal pellet was washed twice in 20 ml of buffer by gentle homogenization, followed by resedimentation at 20,000 *g*. The washed crude microsomal pellet was then dispersed in sucrose-phosphate buffer.

Aliquots of microsomal suspensions (200 μl, equivalent to 300 mg of liver or kidney) were added to 1.6 ml of phosphate-sucrose buffer, or the same volume of liver or kidney cytosol, previously enriched with ^{125}I-T_4 (1 μCi/ml, 0.020 μg/ml) or ^{125}I-T_3 (1.3 μCi/ml, 0.025 μg/ml). Cofactors or chemicals dissolved in 300 μl of buffer or the same volume of buffer alone were then added to attain a final incubation volume of 2.1 ml. The resulting mixtures were incubated for 3 h at 37°C under an atmosphere of N_2. Under these conditions, the generation of ^{125}I-T_3 from ^{125}I-T_4 in mixtures of microsomes in either buffer or cytosol increased progressively over a 4-h incubation period. In all experiments, two types of control vessels were employed, one containing tissue fractions and labeled hormone, but incubated at 0°C, and the other incubated at 37°C, but containing no tissue. Where appropriate, control vessels contained the same chemical or cofactor additives that were present in experimental vessels. No significant degradation of ^{125}I-T_4 or generation of ^{125}I-T_3 was observed in these control preparations. After incubation, vessels were quickly placed on cracked ice, and portions of the complete reaction mixtures were combined with equal volumes of blood bank plasma and stored frozen at −20°C for subsequent analysis by paper chromatography.

Paper chromatography. Frozen reaction mixtures were thawed and mixed thoroughly, and 10-μl aliquots were applied together with carrier compounds to Whatman 3MM (Whatman, Inc., Clifton, N. J.) paper chromatographic strips. The strips were chromatographed in a descending hexane-tertiary amyl alcohol-2 N ammonia (1:10:11) solvent system. Chromatograms were dried; carrier iodothyronines were localized with the aid of ultraviolet light and carrier iodide (I^-) by spraying with palladium chloride. Zones corresponding to the origin and carrier compounds were excised and counted in a gamma scintillation counter. Over 98% of the total radioactivity recovered from chromatograms between the origin and the solvent front was contained in these discrete zones. Values for the percentage generation of labeled products during incubations with ^{125}I-T_4 or ^{125}I-T_3 were corrected for the percentage contamination of these products found in nonmetabolizing control vessels. ^{125}I-T_4 was 96–97% pure, containing 0.3–0.5% ^{125}I-T_3 and 1–2% ^{125}I-I^- as contaminants, whereas ^{125}I-T_3 was 95% pure and contained 2% ^{125}I-I^- as the only definable contaminant.

Protein measurements. The method of Lowry and co-workers (39) was employed to measure protein concentrations in preparations of either cytosol or microsomes that had been dispersed in 0.1% deoxycholate.

Statistical analysis. All experiments were performed three or more times, with close concurrence of the results obtained. Hence, results were pooled and subjected to statistical analysis by the Student's *t* test when a single experimental variable was studied. When more than two

[2] Phenolic-ring ^{125}I-labeled L-thyroxine (^{125}I-T_4), 50–70 μCi/μg sp act, and 3,5,3′-L-triiodothyronine (^{125}I-T_3), 50–75 μCi/μg, were purchased from Abbott Laboratories (Chicago, Ill.). Crystalline T_4 and T_3, NAD and NADH, NADP, NADPH, isocitric acid, isocitric acid dehydrogenase, GSH, GSSG, and diamide (azodicarboxylic acid-*bis*-dimethylamide) were all purchased from Sigma Chemical Co. (St. Louis, Mo.).

[3] Pelleted Laboratory Chow, RMH 1000, was purchased from Agway-Country Foods (Syracuse, N. Y.).

variables were studied, the significance of differences between results in specific groups was assessed by analysis of variance, followed by Duncan's multiple range test (40).

RESULTS

In all of the studies performed, only ^{125}I-labeled T_3, I^-, origin material (OM), and a compound with the chromatographic mobility of 3,5,3′,5′-tetraiodothyroacetic acid, appeared as products of ^{125}I-T_4 metabolism. Labeled reverse T_3 was not detected, presumably because of its rapid degradation. The principle focus of these investigations was the formation of T_3 from T_4; hence, only results concerning the quantity of T_4 degraded and the quantities of T_3 and I^- formed will be discussed. ^{125}I-Labeled origin material and carrier 3,5,3′,5′-tetraiodothyroacetic acid never accounted for more than a few percent of total ^{125}I on final chromatograms, and results with respect to these compounds will not be presented or discussed.[4]

T_3 generation from T_4 by microsomes incubated in buffer. In a series of 18 experiments, the proportion of ^{125}I-T_3 present after incubation of microsomes in buffer was compared with that found after incubation of tissue-free controls. Values averaged 1.4±0.6% (mean±SE) of added T_4 in the presence of microsomes and 0.6±0.1% in the absence of microsomes ($P < 0.001$). This indicates that very slight, but significant, generation of T_3 takes place in microsomes incubated in buffer alone.

Effects of fasting. When incubated in buffer, liver microsomes from fed and from fasted rats generated similar quantities of ^{125}I-T_3 from ^{125}I-T_4. When liver cytosol from fed rats was substituted for buffer, generation of ^{125}I-T_3 was increased approximately threefold, regardless of whether microsomes had been obtained from fed or fasted rats (Table I). Moreover, when liver cytosol from fed rats was replaced by

[4] An Appendix containing a complete tabulation of all the results of the experiments presented in this manuscript, together with statistical analyses of the significance of differences between means for all functions measured among all experimental groups, has been deposited with the National Auxiliary Publications Service (NAPS) document 03436. This information may be ordered from ASIS/NAPS, Microfiche Publications, P.O. Box 3513 Grand Central Station, New York 10017. Remit in advance $3.00 for microfiche copy, or for photocopy, $5.00 up to 20 pages plus 25¢ for additional pages. Checks should be made payable to Microfiche Publications.

TABLE I

Effect of Fasting on the Metabolism of ^{125}I-T_4 in Mixtures of Microsomes and Buffer and Microsomes and Cytosols from Rat Liver

Microsome source	Cytosol source	Experiments*	^{125}I-T_4 degradation (percent added T_4)		^{125}I-T_3 formation (percent added T_4)		^{125}I-I formation (percent added T_4)	
			Mean±SE	Significance‡	Mean±SE	Significance	Mean±SE	Significance
		n						
(*a*) Fed	(Buffer)	8	7.0±1.1	NS	1.1±0.1	NS	4.4±1.1	NS
				a vs. *b*		*a* vs. *b*		*a* vs. *b*
				c vs. *d*		*c* vs. *d*		*a* vs. *e*
(*b*) Fasted	(Buffer)	8	6.1±2.0	*e* vs. *f*	1.1±0.1	*e* vs. *f*	3.8±0.7	*b* vs. *f*
								c vs. *e*
				$P < 0.01$		$P < 0.01$		*c* vs. *f*
								e vs. *f*
(*c*) Fed	Fed	13	16.9±1.1	*a* vs. *c*	3.9±0.2	*a* vs. *c*	8.8±0.7	
				a vs. *e*		*a* vs. *e*		$P < 0.05$
				b vs. *d*		*b* vs. *d*		
				b vs. *f*		*b* vs. *f*		*c* vs. *f*
(*d*) Fasted	Fed	13	17.7±1.4	*c* vs. *e*	3.4±0.2	*c* vs. *e*	9.4±1.0	
				c vs. *f*		*c* vs. *f*		$P < 0.01$
				d vs. *f*		*d* vs. *f*		
								a vs. *c*
(*e*) Fed	Fasted	13	12.0±1.1		2.6±0.3		6.8±0.9	*b* vs. *d*
								d vs. *f*
(*f*) Fasted	Fasted	13	11.5±1.3		2.3±0.2		5.8±1.0	

* Number of separate experiments performed for each experimental group.

‡ Levels of statistical significance of differences between values among various experimental groups. Analyses shown only for those comparisons considered to be important.

cytosol from fasted rats, generation of ^{125}I-T_3 was greatly decreased, and again values obtained with microsomes from fed and fasted animals did not differ significantly. However, even cytosol from fasted rats provided significantly greater support of T_3-neogenesis than buffer alone did. As with T_3 formation, the degradation of T_4 and formation of I^- were least in incubations containing buffer, greater in those containing cytosol from fasted rats, and greatest in those containing cytosol from fed rats.

As judged from six experiments, livers from fed and fasted animals did not differ significantly in the protein concentrations (milligrams per gram wet liver weight) of their respective microsomes (11.6±0.6 vs. 10.5±1.1) or cytosols (56.7±3.8 vs. 62.7±2.9).

Effects of glucose replacement. Comparable experiments were performed with varying mixtures of microsomes and cytosols derived from fed or glucose-replaced rats because previous studies had shown that glucose administration prevented the decreased T_3-neogenesis otherwise found in homogenates and slices of liver from fasted animals. In these experiments, T_3 formation was again greater in the presence of cytosol than of buffer and, with respect to either microsomal activity or cytosolic stimulation, there was no difference between preparations from chow-fed and glucose-replaced animals (data not shown).

Effects of fasting in varying mixtures of microsomes and cytosols from liver and kidney. In earlier studies, we have demonstrated that after a 48-h period of fasting, T_3 formation is diminished in preparations from liver, but not from kidney. This observation served as the rationale for assessing the effect of fasting on T_3 generation in varying mixtures of liver and kidney microsomes and cytosols from fed and fasted animals (Table II). In the presence of liver cytosol from fed rats, kidney microsomes from fed and fasted rats did not differ in T_3-neogenetic activity. The same was true when kidney microsomes were incubated in liver cytosol from fasted animals, though the level of activity was significantly less.

Converse experiments were performed in which liver microsomes from fed or fasted animals were incubated with kidney cytosol from the same two sources. T_3-neogenesis in the four different combinations was essentially the same. This was also true when

TABLE II

Effect of Fasting on the Metabolism of ^{125}I-T_4 in Mixtures of Microsomes and Cytosols from Livers and Kidneys of Rats

Microsome source	Cytosol source	Experiments*	^{125}I-T_4 degradation (percent added T_4) Mean±SE	Significance‡	^{125}I-T_3 formation (percent added T_4) Mean±SE	Significance	^{125}I-I formation (percent added T_4) Mean±SE	Significance
Kidney microsomes and liver cytosols								
		n						
(*a*) Fed, kidney	Fed, liver	7	12.5±2.5	NS	2.2±0.1	NS	5.1±1.4	NS
(*b*) Fasted, kidney	Fed, liver	7	9.5±1.1	*a* vs. *b* *a* vs. *c*	2.3±0.4	*a* vs. *b* *c* vs. *d*	2.8±0.9	*a* vs. *b* *a* vs. *c*
(*c*) Fed, kidney	Fasted, liver	7	10.6±1.9	*b* vs. *d* *c* vs. *d*	1.3±0.2	$P < 0.05$ *a* vs. *c*	4.3±0.8	*b* vs. *d* *c* vs. *d*
(*d*) Fasted, kidney	Fasted, liver	7	9.5±1.8		0.8±0.2	$P < 0.01$ *b* vs. *d*	4.1±1.1	
Liver microsomes and kidney cytosols								
(*a*) Fed, liver	Fed, kidney	9	14.1±1.9	NS	2.4±0.3	NS	5.4±1.6	NS
(*b*) Fasted, liver	Fed, kidney	9	17.3±1.5	*a* vs. *b* *a* vs. *c*	2.2±0.2	*a* vs. *b* *a* vs. *c*	4.6±1.1	*a* vs. *b* *a* vs. *c*
(*c*) Fed, liver	Fasted, kidney	8	15.6±2.8	*b* vs. *d* *c* vs. *d*	1.8±0.3	*b* vs. *d* *c* vs. *d*	5.1±2.1	*b* vs. *d* *c* vs. *d*
(*d*) Fasted, liver	Fasted, kidney	8	17.7±1.9		1.9±0.2		3.7±1.3	

* Number of separate experiments performed for each experimental group.

‡ Levels of statistical significance of differences between values among various experimental groups. Analyses shown only for those comparisons considered to be important.

kidney microsomes from fed or fasted rats were mixed with kidney cytosol from the same two sources (data not shown).

Effect of NADPH. Experiments were conducted to assess the effect of adding an NADPH-generating system to liver microsomes from fed or fasted rats incubated either in buffer or in liver cytosols from fed or fasted animals (Table III).[5] In the presence of buffer, T_3-neogenesis by microsomes was not enhanced significantly by NADPH. Neither did NADPH further increase the stimulatory effect of cytosol from animals that had been fed. However, NADPH enrichment of cytosol from fasted animals restored microsomal T_3-neogenesis to the greater activity seen in the presence of cytosol from fed animals. In none of the foregoing mixtures did NADH significantly influence T_3-neogenesis (data not shown). In mixtures of microsomes with cytosol from fed animals, NADPH consistently appeared to decrease both ^{125}I-T_4 degradation and ^{125}I-I^- formation, though the differences were not statistically significant by the methods of analysis employed.

Effect of GSH. Previous studies have shown that various mercaptans stimulate the T_3-neogenesis in liver homogenates (41) and microsomes (42–44). Therefore, experiments were performed to assess the effect of GSH in microsomes incubated in buffer or in cytosols obtained from either fed or fasted rats (Table IV). In these and ensuing studies, all microsomes were derived from the livers of fed rats.

Liver cytosol from fed animals was again found to be more supportive of T_3-neogenesis than was cytosol from fasted animals. In the presence of cytosol from fed animals, addition of GSH (5 mM) increased T_3 formation slightly, but significantly. Similar enrichment of

[5] Although the experiments shown in Table III involved studies of microsomes from fasted as well as fed animals, only results obtained in microsomes from fed animals are shown, as no difference in the responsiveness of microsomes from fed or fasted animals was seen in these or any of the other experiments described.

TABLE III

Effect of NADPH on the Metabolism of ^{125}I-T_4 in Mixtures of Liver Microsomes from Fed Rats with Buffer or Liver Cytosols from Fed and Fasted Rats

			^{125}I-T_4 degradation (percent added T_4)		^{125}I-T_3 formation (percent added T_4)		^{125}I-I formation (percent added T_4)	
Cytosol source	Additive*	Experiments‡	Mean±SE	Significance§	Mean±SE	Significance	Mean±SE	Significance
		n						
(*a*) (Buffer)	—	5	6.4±1.5	NS	1.1±0.2	NS	3.7±1.5	NS
				a vs. *b*		*a* vs. *b*		*a* vs. *b*
				c vs. *d*		*c* vs. *d*		*a* vs. *c*
(*b*) (Buffer)	NADPH	5	5.8±1.5	*c* vs. *e*	1.5±0.2	*c* vs. *f*	3.2±1.3	*a* vs. *d*
				d vs. *f*		*d* vs. *f*		*a* vs. *e*
				e vs. *f*				*b* vs. *d*
						$P < 0.05$		*b* vs. *f*
(*c*) Fed	—	6	16.2±1.7	$P < 0.05$	4.1±0.3		7.4±0.9	*c* vs. *d*
						a vs. *e*		*c* vs. *e*
				a vs. *d*		*e* vs. *f*		*c* vs. *f*
				a vs. *e*				*d* vs. *f*
(*d*) Fed	NADPH	6	11.6±2.2	*b* vs. *d*	3.7±0.5	$P < 0.01$	4.9±1.4	*e* vs. *f*
				b vs. *f*				
				c vs. *f*		*a* vs. *c*		
						a vs. *d*		
(*e*) Fasted	—	6	12.1±1.8	$P < 0.01$	2.6±0.2	*b* vs. *d*	6.2±1.6	
						b vs. *f*		
				a vs. *c*		*c* vs. *e*		
(*f*) Fasted	NADPH	6	11.1±1.9		3.7±0.6		6.0±1.4	

* NADPH added as an NADPH-generating system: isocitric acid dehydrogenase, 1 mg/ml, isocitric acid, 20 mM, and NADP, 0.1 mM.

‡ Number of separate experiments performed for each experimental group.

§ Levels of statistical significance of differences between values among various experimental groups. Analyses shown only for those comparisons considered to be important.

TABLE IV

Effect of GSH on the Metabolism of ^{125}I-T_4 in Mixtures of Liver Microsomes from Fed Rats with Buffer or Liver Cytosols from Fed and Fasted Rats

Cytosol source	Additive*	Experiment‡	^{125}I-T_4 degradation (percent added T_4)		^{125}I-T_3 formation (percent added T_4)		^{125}I-I formation (percent added T_4)	
			Mean±SE	Significance§	Mean±SE	Significance	Mean±SE	Significance
		n						
(*a*) Fed	—	6	16.5±1.4	NS	4.5±0.2	NS	8.0±0.9	NS
				a vs. *c*		*a* vs. *d*		*a* vs. *b*
				a vs. *d*				*a* vs. *c*
(*b*) Fasted	—	6	10.9±0.9	*c* vs. *d*	2.3±0.4	$P < 0.01$	4.8±1.1	*a* vs. *d*
				c vs. *f*				*b* vs. *d*
						a vs. *b*		*b* vs. *e*
				$P < 0.05$		*a* vs. *c*		*c* vs. *d*
(*c*) Fed	GSH	6	18.5±1.4		5.9±0.3	*a* vs. *e*	9.4±1.0	*c* vs. *f*
				b vs. *d*		*b* vs. *d*		
						b vs. *e*		$P < 0.05$
				$P < 0.01$		*c* vs. *d*		
(*d*) Fasted	GSH	6	15.2±1.1		4.9±0.4	*c* vs. *f*	7.5±1.3	*a* vs. *e*
				a vs. *b*		*d* vs. *f*		*d* vs. *f*
				a vs. *e*		*e* vs. *f*		
				b vs. *e*				$P < 0.01$
(*e*) (Buffer)	—	5	5.0±0.9	*d* vs. *f*	0.8±0.2		3.1±0.8	
				e vs. *f*				*e* vs. *f*
(*f*) (Buffer)	GSH	5	22.2±2.3		8.6±0.6		12.4±2.1	

* GSH, 5 mM.

‡ Number of separate experiments performed for each experimental group.

§ Levels of statistical significance of differences between values among various experimental groups. Analyses shown only for those comparisons considered to be important.

preparations containing cytosol from fasted animals increased T_3 formation more markedly, and values were then comparable to those seen in mixtures containing cytosol from fed animals. T_4 degradation was increased significantly by GSH in mixtures containing cytosol from fasted animals, but not from fed animals.

In preparations containing microsomes in buffer, GSH increased T_3 generation >10-fold, and both the disappearance of T_4 and generation of iodide were also markedly enhanced. This finding was in marked contrast to that obtained with NADPH, which had no effect on microsomal T_3-neogenesis in the absence of cytosol.

The effects of GSSG (5 mM) in these systems were quite distinct from those of GSH. Thus, in four experiments, values for percentage microsomal generation of T_3 in the presence of cytosol from fed animals were: (*a*) 3.1±0.4 in the absence of any additions; (*b*) 4.3±0.4 in the presence of GSH; and (*c*) 2.0±0.2 in the presence of GSSG (*a* vs. *b*, $P < 0.05$; *b* vs. *c*, $P < 0.01$; *a* vs. *c*, NS). Corresponding values obtained when microsomes were incubated in buffer, rather than cytosol, were: (*a*) 0.5±0.1; (*b*) 7.9±0.3; and (*c*) 0.4±0.1 (*a* vs. *b*, $P < 0.01$; *b* vs. *c*, $P < 0.01$; *a* vs. *c*, NS).

Effect of diamide. To determine the relationship between the actions of NADPH and GSH within these systems, experiments were performed to ascertain the effects of the sulfhydryl oxidant, diamide (45), in microsomal preparations suspended in either cytosol or buffer (Table V). As previously seen, T_3 formation in the presence of cytosol from fasted rats was enhanced by the addition of GSH. In this system, diamide greatly decreased T_3 formation and blocked the stimulatory effect of added GSH. In the presence of buffer, rather than cytosol, values for microsomal T_3 generation were low and were unaffected by diamide. GSH was once again seen to stimulate enzyme activity >10-fold and, as expected, diamide prevented this effect. Effects of GSH and diamide, separately and in combination, on T_4 degradation and I^- formation were parallel to their effects on T_3-neogenesis.

TABLE V

Effect of Diamide and Cofactors on the Metabolism of ^{125}I-T_4 in Mixtures of Liver Microsomes from Fed Rats with Buffer or Cytosols from Livers of Fed and Fasted Rats

Cytosol source	Additive*	Experiment‡	^{125}I-T_4 degradation (percent added T_4)		^{125}I-T_3 formation (percent added T_4)		^{125}I-I formation (percent added T_4)	
		n	Mean±SE	Significance§	Mean±SE	Significance	Mean±SE	Significance
Effect of diamide and GSH								
(*a*) Fasted	—	6	8.1±1.7	NS	1.6±0.4	NS	3.9±1.2	NS
(*b*) Fasted	GSH	6	12.3±1.0	*a* vs. *c* *c* vs. *d*	3.9±0.3	*c* vs. *d* *c* vs. *e*	6.5±0.7	*c* vs. *d* *c* vs. *e*
(*c*) Fasted	Diamide	6	5.0±0.5	*c* vs. *e* *e* vs. *f*	0.4±0.3	*e* vs. *f* *e* vs. *h*	1.2±0.3	*e* vs. *f* *e* vs. *h*
(*d*) Fasted	GSH and diamide	6	4.6±0.7	*e* vs. *h* *f* vs. *h*	0.3±0.1	*f* vs. *h*	1.9±0.4	*f* vs. *h*
(*e*) (Buffer)	—	5	5.1±1.1	$P < 0.05$	0.4±0.1	$P < 0.01$	3.6±0.9	$P < 0.05$
(*f*) (Buffer)	Diamide	5	6.2±0.9	*a* vs. *b*	0.5±0.1	*a* vs. *b* *a* vs. *c*	3.5±0.6	*a* vs. *b* *a* vs. *c*
(*g*) (Buffer)	GSH	5	19.4±1.4	$P < 0.01$	6.5±0.6	*b* vs. *d* *e* vs. *g*	9.5±1.5	*b* vs. *d*
(*h*) (Buffer)	GSH and diamide	5	8.3±1.2	*b* vs. *d* *e* vs. *g* *g* vs. *h*	0.7±0.1	*g* vs. *h*	5.7±0.8	$P < 0.01$ *e* vs. *g* *g* vs. *h*
Effect of diamide and NADPH								
(*a* Fed	—	5	13.6±2.5	NS	3.6±0.6	NS	6.1±1.6	NS
(*b*) Fasted	—	5	8.4±2.0	*a* vs. *f* *b* vs. *d*	1.7±0.5	*b* vs. *d* *b* vs. *h*	4.1±1.4	*a* vs. *b* *a* vs. *c*
(*c*) Fed	Diamide	5	8.3±1.8	*b* vs. *h* *c* vs. *g*	0.4±0.1	*c* vs. *g* *d* vs. *h*	3.4±1.3	*a* vs. *f* *b* vs. *d*
(*d*) Fasted	Diamide	5	5.1±0.6	*d* vs. *h* *e* vs. *f*	0.4±0.03	*e* vs. *f* *g* vs. *h*	1.3±0.4	*b* vs. *h* *c* vs. *g*
(*e*) Fed	NADPH	5	11.6±1.1	*g* vs. *h*	4.6±0.4	$P < 0.01$	5.9±0.7	*d* vs. *h* *g* vs. *h*
(*f*) Fasted	NADPH	5	14.0±1.0	$P < 0.05$	5.7±0.4	*a* vs. *b*	7.3±0.9	
(*g*) Fed	Diamide and NADPH	5	4.4±1.3	*a* vs. *b*	1.3±0.6	*a* vs. *c*	2.1±0.7	$P < 0.05$
(*h*) Fasted	Diamide and NADPH	5	4.9±1.6	*a* vs. *c* *b* vs. *f* $P < 0.01$ *a* vs. *g* *e* vs. *g* *f* vs. *h*	1.1±0.6	*a* vs. *f* *a* vs. *g* *b* vs. *f* e vs. *g* *f* vs. *h*	1.9±1.1	*a* vs. *g* *b* vs. *f* *e* vs. *g* $P < 0.01$ *f* vs. *h*

* GSH, 5 mM, diamide, 10 mM, and NADPH as a NADPH-generating system: isocritic acid dehydrogenase, 1 mg/ml, isocritic acid, 20 mM, and NADP, 0.1 mM.

‡ Number of separate experiments performed for each experimental group.

§ Levels of statistical significance of differences between values among various experimental groups. Analyses shown only for those comparisons considered to be important.

As observed in previous experiments, NADPH had no significant effect on microsomal T_3-neogenesis in the presence of cytosol from fed animals, but restored values in the presence of fasted cytosol to those seen in the former group. With both types of cytosol, with or without NADPH, T_3-neogenesis was substantially inhibited by diamide. In all combinations tested, diamide diminished, but did not abolish, T_4 degradation and the generation of I^-.

Effect of cofactors on the metabolism of ^{125}I-T_3. Relative to rates of ^{125}I-T_4 degradation and ^{125}I generation therefrom, rates of ^{125}I-T_3 degradation under comparable conditions were quite low. In microsomal preparations incubated with cytosol from fed animals, NADPH decreased both T_3 degradation and I^- generation, whereas GSH had little effect (Table VI). T_3 metabolism was slower in mixtures containing cytosol from fasted than from fed rats, and no significant changes were detected after the addition of cofactors to cytosol from fasted animals. The effects of NADPH and possibly GSH on T_3 metabolism by microsomes incubated in buffer differed from their effects on T_3-neogenesis under these conditions. Thus, NADPH had an inhibitory effect on T_3 degradation, though it had not affected T_3 formation under these conditions. Moreover, although GSH enhanced T_3 degradation, as it had in the case of T_4, the proportionate effect was far less in the case of T_3.

DISCUSSION

In a previous report (33), we presented evidence that T_3-neogenesis in slices or homogenates of rat liver provides a reliable model of this process in man. Among

TABLE VI

Effects of Cofactors on the Metabolism of ^{125}I-T_3 in Mixtures of Liver Microsomes from Fed Rats with Buffer or Liver Cytosols from Fed and Fasted Rats

			^{125}I-T_3 degradation (percent added T_3)		^{125}I-I formation (percent added T_3)	
Cytosol source	Additive*	Experiments‡	Mean±SE	Significance§	Mean±SE	Significance
		n				
(*a*) Fed	—	8	6.7±1.6	NS	3.5±1.0	NS
(*b*) Fed	NADPH	8	1.7±0.2	*a* vs. *c* *a* vs. *f* *b* vs. *e* *d* vs. *e*	1.7±0.2	*a* vs. *d* *a* vs. *e* *a* vs. *f* *b* vs. *e*
(*c*) Fed	GSH	5	8.6±1.9	*d* vs. *f* *e* vs. *f*	5.9±1.4	*d* vs. *e* *d* vs. *f* *e* vs. *f*
(*d*) Fasted	—	6	3.3±0.8	$P < 0.05$	1.6±0.4	$P < 0.05$
(*e*) Fasted	NADPH	6	2.1±0.4	*a* vs. *d* *c* vs. *f*	1.5±0.3	*a* vs. *b* *a* vs. *c*
(*f*) Fasted	GSH	4	4.5±1.2	$P < 0.01$	2.3±0.8	*a* vs. *g* *g* vs. *h*
(*g*) (Buffer)	—	8	2.6±0.4	*a* vs. *b* *a* vs. *e* *a* vs. *g* *g* vs. *h* *g* vs. *i*	1.8±0.3	$P < 0.01$ *c* vs. *f* *g* vs. *i*
(*h*) (Buffer)	NADPH	8	0.9±0.2	*h* vs. *i*	0.9±0.2	*h* vs. *i*
(*i*) (Buffer)	GSH	6	4.1±0.4		3.7±0.5	

* GSH, 5 mM, NADPH added as an NADPH-generating system: isocitric acid dehydrogenase, 1 mg/ml isocitric acid, 20 mM, and NADP, 0.1 mM.

‡ Number of separate experiments performed for each experimental group.

§ Levels of statistical significance of differences between values among various experimental groups. Analyses shown only for those comparisons considered to be important.

the evidence in support of this suggestion was the finding that a 48-h fast in the rat greatly reduced T_3-neogenesis from T_4 in liver preparations in vitro, and this finding seemed to correlate with the known reduction in overall T_3-neogenesis that occurs during periods of fasting in man.

In the present studies, we have sought to determine the factors responsible for the decrease in hepatic formation of T_3 from T_4 occasioned by fasting and to elucidate thereby certain of the factors that regulate the activity of the 5′-monodeiodinating mechanism for T_4. To do this, we have examined the T_3-neogenetic activity of particular hepatic subcellular fractions, alone and in combination, from normal and fasted animals, with and without added cofactors. Specifically, we have examined the activity of crude microsomal preparations, in which the 5′-monodeiodinating enzyme is thought to reside (42, 43) and have studied the influence thereon of hepatic cytosol, a subcellular fraction virtually devoid of intrinsic deiodinase activity. Our observations are consonant with others that indicate that crude microsomal preparation is richest in 5′-monodeiodination for T_4 (42–44), because under any given condition this fraction was more active on a unit protein basis than nuclear, mitochondrial, or cytosolic fractions were (data not shown). Distinct, though low, activity was present in microsomes incubated in buffer, because values for T_3 generation, though small, consistently exceeded those found in tissue-free or unincubated controls. The data further provided clear evidence of the existence of stimulatory cofactors within the cytosol, because incubation of microsomes in cytosol invariably yielded greater proportions of T_3 than were found during incubations of microsomes in buffer alone.

Several lines of evidence indicated that the defective hepatic T_3-neogenesis associated with a 48-h period of fasting was the result of a cytosolic, rather than a microsomal, abnormality. Microsomes from liver of fed and fasted rats displayed similar T_3-neogenetic activity, which was lowest when incubated in buffer, greater when cytosol from fasted animals was employed, and greatest when cytosol from fed animals was the suspending vehicle.

Further evidence to this point was provided by experiments in which microsomes and cytosols from liver of fed or fasted animals were variously mixed with analogous fractions from kidney, previous studies in homogenates and slices having shown that renal T_3-neogenesis in vitro is unaffected by a 48-h fast. When only renal microsomes and cytosols were used, T_3 formation from T_4 was essentially the same in all four combinations of microsomes and cytosols from fed and fasted animals (data not shown). This finding tends to validate the use of the recombination system as a reflection of T_3-neogenesis in unfractionated homogenates. Hence, the fact that T_3 formation was unaffected by the nutritional state of the donor of hepatic microsomes or of renal cytosol provided further indication of the normality of microsomal enzyme in the liver of the fasted rat. In contrast, cytosol from livers of fasted rats was consistently less supportive of T_3-neogenesis by renal microsomes than was hepatic cytosol from fed rats.

From the foregoing observations we would conclude that in the rat fasted for 48 h the defect in hepatic T_3-neogenesis resides in deficient cytosolic support, rather than an intrinsic abnormality in the 5′-monodeiodinating enzyme. From studies in livers of animals fasted for 72 h, Kaplan (44) has concluded that fasting is associated with both cytosolic and microsomal abnormalities. We have confirmed that this is the case in animals fasted for this period, but our own data indicate that the microsomal abnormality is secondary to the hypothyroidism that supervenes in the fasted animal, because it is prevented by the administration of physiological replacement doses of T_4 during the period of fasting.[6] It was for this reason that all animals in the present studies, controls as well as those fasted, were given exogenous T_4.

As anticipated from our previous observations in whole homogenates or slices, abnormalities in the hepatic cytosol were prevented by giving the donor rats 25% glucose in the drinking water during the period of fasting. These data, together with our earlier demonstration of diminished hepatic T_3-neogenesis in experimental diabetes and its reversal by insulin, strongly suggested that in the rat the hepatic generation of T_3 from T_4 is closely linked to the rate of glucose utilization. This, in turn, provided a clue to the nature of the abnormality in liver cytosol produced by fasting. Our attention was directed to the role of NADPH because the activity of the hexosemonophosphate shunt, and hence the rate of the NADP reduction, is markedly impaired by fasting (46). Moreover, NADPH serves as a donor of hydrogen in several reductive syntheses, such as lipogenesis (47) and A-ring reduction of corticosteroids (48, 49), and 5′-monodeiodination of T_4 is also a reductive process involving substitution of hydrogen for iodine in the phenolic ring of the T_4 molecule. Most importantly, the concentration of NADPH has been shown to be reduced in the liver of fasted rats (46). As might be expected from these considerations, addition of an NADPH-generating system to hepatic cytosol from fasted animals restored the supporting activity with respect to T_3-neogenesis to a level comparable to that

[6] Balsam, A., F. Sexton, and S H. Ingbar. Unpublished observations.

of cytosol from fed animals, regardless of the nutritional state of the donor of hepatic microsomes.[7]

Several studies have indicated that compounds containing reduced sulfhydryl groups, such as GSH and dithiothreitol, are stimulatory to T_3-neogenesis (41, 42, 44, 50, 51), a finding that we have presently confirmed with respect to liver. Inasmuch as fasting decreases the hepatic GSH concentration in rats (50, 52, 53), we would suggest that a lowering of cytosolic GSH is a second abnormality responsible for decreased T_3-neogenesis in the liver of fasted animals. Consistent with this suggestion is the observation that the impaired supporting activity of cytosol from fasted rats was restored to normal by the addition of physiological concentrations of GSH. Hence, it appears that with respect to T_3-neogenesis, there are two factors deficient in the hepatic cytosol of fasted rats, NADPH and GSH. It seems likely, in addition, that the two abnormalities are at least partly related because NADPH is the cofactor in the reduction of GSSG by the enzyme, glutathione reductase. This suggestion is consonant with our finding that NADPH acted only in the presence of cytosol, whereas GSH acted not only in the presence of cytosol, but also when added directly to buffer.

Activity of the microsomal enzyme incubated in buffer was seemingly unaffected by the addition of diamide. Although this conclusion must be considered tentative because of the low activity of microsomes in buffer alone, this finding would suggest that the 5'-monodeiodinase does not depend upon intact sulfhydryl groups within its molecule. If that is indeed the case, then the remarkable direct stimulation of T_3-neogenesis by microsomes in buffer that GSH produces cannot be ascribed to reduction of intramolecular sulfhydryl groups; it suggests instead that the enzyme may act as a GSH transhydrogenase, analogous to that involved in the metabolism of insulin (54, 55).

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[7] Although NADPH did reduce the extent of T_3 degradation under conditions in which it enhanced net T_3-neogenesis, the former effect was not sufficient in itself to explain the latter. Thus, when added to cytosol from fasted animals, NADPH increased T_3-neogenesis by approximately half. Under similar incubation conditions, the mean percent of ^{125}I-T_3 that remained undegraded was increased by NADPH only from 96.7 to 97.9%.

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