CLINICAL RESEARCH

Raised plasma glutathione S-transferase values in hyperthyroidism and in hypothyroid patients receiving thyroxine replacement: evidence for hepatic damage

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Abstract

Using plasma glutathione S-transferase measurements hepatocellular integrity was assessed in groups of hyperthyroid and hypothyroid patients before and after treatment.

Ten of 14 hyperthyroid patients had clearly raised plasma glutathione S-transferase values at presentation and in each patient treatment with either iodine-131 or carbimazole resulted in a significant fall in glutathione S-transferase. The eight hypothyroid patients had normal glutathione S-transferase values at presentation and all showed a significant increase in these after thyroxine replacement therapy. In three of these patients in whom standard doses of replacement therapy were associated with a raised free thyroxine (T4) concentration but normal total and free triiodothyronine (T3) values glutathione S-transferase was increased. Similar though less consistent changes were seen in the results of standard chemical tests of liver function.

It is concluded that hyperthyroidism may produce subclinical liver damage in a high proportion of patients and that this resolves with effective treatment. More important, the data suggest that hypothyroid patients receiving thyroxine replacement therapy may have similar subclinical liver damage.

Patients receiving thyroxine should be monitored by the measurement of free, not total hormone concentrations, and in those in whom free T4 is raised the dose of thyroxine should be reduced. It would also be expedient to include periodic biochemical assessment of liver function in patients receiving thyroxine.

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Introduction

Before the advent of effective treatment for hyperthyroidism serious hepatobiliary complications were commonly associated with the disease. Liver biopsy samples taken from hyperthyroid patients often showed morphological changes which included glycogen depletion, fatty change, and cirrhosis. These associated hepatic problems have been attributed to many factors, including cardiac failure, infection, hypoxia, and malnutrition, and are not thought to be due to a direct effect of thyroid hormones on the liver.[1]

Prolonged and severe hyperthyroidism now occurs only occasionally and, as a consequence, severe hepatobiliary dysfunction associated with hyperthyroidism is rare. There is, however, evidence that hyperthyroidism may still produce associated minor hepatic abnormalities, and electron microscopy of liver biopsy samples shows non-specific changes in the hepatocyte organelles.[2] Biochemical evidence of hepatic dysfunction may still be seen in some patients with hyperthyroidism. In one study retention of sulphobromophthalein was abnormal in 8% of patients, and abnormalities in bilirubin and alanine aminotransferase values also were observed, but less frequently.[3]

There is little evidence to suggest that hypothyroidism affects liver function, but these patients will subsequently receive levothyroxine replacement therapy. As a result many will have raised plasma total and free thyroxine (T4) concentrations with normal total and free triiodothyronine (T3) concentrations.[4] Of particular interest is the question whether these patients, who appear euthyroid clinically, have tissue hyperthyroidism.[5] Measurement of the changes which occur in cardiac and pituitary response in these patients suggests that tissue hyperthyroidism does result from this treatment.[5] Nevertheless, thyroxine replacement therapy has not been shown to have a detrimental effect on hepatic function.

The measurement of plasma glutathione S-transferase by radioimmunoassay offers an extremely sensitive method of investigating hepatocellular damage, both in animals and man.[6-9] These measurements are non-invasive and the results correlate well with the histological findings in the liver.[7] In drug induced liver damage plasma glutathione S-transferase measurements appear to be some 10 times more sensitive at detecting damage than measurements of aminotransferase activities.[8]

We report the use of plasma glutathione S-transferase measurements and standard chemical tests of liver function to investigate the hepatocellular integrity of patients with thyroid diseases. We have also examined the effect of thyroxine replacement on hepatocellular integrity in hypothyroid patients.

Patients and methods

HYPERTHYROID PATIENTS

We studied two groups of patients with hyperthyroidism. The first group consisted of five women aged 46-75 years (cases 1-5; see table I) who were treated with iodine-131. All had raised plasma concentrations of total T4 and total T3 at presentation, and all showed a basally suppressed thyroid stimulating hormone concentration which did not rise by more than 1 mU/l 20 minutes after an intravenous injection of 200 μg thyrotrophin releasing hormone.

The second group consisted of nine younger women (aged 19-45 years; cases 6-14) in whom a study had been undertaken to assess the merits of different treatment schedules with carbimazole. These schedules were (*a*) 15 mg carbimazole three times a day for one month, subsequently reduced in the light of clinical and biochemical response, and (*b*) administration of a continuous blocking dose of 15 mg carbimazole three times a day with triiodothyronine replacement (20 μg thrice daily) being added after roughly one month to prevent hypothyroidism. As in the first group, all patients had raised total T4 and total T3 concentrations at presentation with a lack of response of thyroid stimulating hormone to injection of thyrotrophin releasing hormone.

In all patients blood was taken at presentation and plasma stored at −20°C for the subsequent measurement of total and free T4 values, total and free T3, glutathione S-transferase, aspartate aminotransferase, γ-glutamyltransferase, and alkaline phosphatase. In the first group of patients a second sample was taken when they had been rendered euthyroid. In the patients treated with carbimazole more frequent sampling was employed.

HYPOTHYROID PATIENTS

The hypothyroid group of patients comprised eight women aged 20-64 years (cases 15-22) presenting with primary atrophic (four patients) or Hashimoto's hypothyroidism. Each patient was assessed at diagnosis and at intervals from three to nine months after beginning thyroxine replacement therapy (see table II). One patient (case 15) was taking the combined oral contraceptive pill.

MEASUREMENTS

Concentrations of total T4 and total T3 were measured by radioimmunoassay using a double antibody system.[10] Free T4 and free T3 concentrations were measured with commercial kits (Amersham International PLC). Reference ranges derived from 98 clinically and biochemically euthyroid patients were: total T4, 65-145 nmol/l (5·1-11·3 μg/100 ml); total T3, 1·2-2·8 nmol/l (0·8-1·8 ng/ml); free T4, 10-22 pmol/l (7·8-17·1 pg/ml); free T3, 4·0-7·8 pmol/l (2·6-5·1 pg/ml).

Plasma glutathione S-transferase was measured by radioimmunoassay.[11] The concentration of hepatic glutathione S-transferase B_1B_1[12] (previously referred to as basic glutathione S-transferase[9]) was measured with a specific antiserum which showed no cross reactivity with the anionic forms of glutathione S-transferase found in red cells, muscle, or lung. The upper limit of the reference range for glutathione S-transferase B_1B_1 was 4·0 μg/l and was derived from 135 blood donors and 40 laboratory volunteers.

The activities of alkaline phosphatase and γ-glutamyltransferase in plasma were measured by a sequential multiple analysis with computer system (SMAC II, Technicon Instrument Corporation, Basingstoke). The activity of aspartate aminotransferase was measured with a centrifugal fast analyser and a kit method (Merckotest A, E Merck, Darmstadt).

The between and within batch coefficient of variation was <9% for each assay.

Statistical analysis was by Wilcoxon's matched pairs test.

Results

HYPERTHYROID PATIENTS GIVEN ^{131}I

Three of the five patients treated with ^{131}I initially had raised plasma glutathione S-transferase values. After treatment all showed a significant ($p < 0.05$) fall in values to within the reference range

TABLE I—*Liver function values in hyperthyroid patients before and after ^{131}I or carbimazole treatment and in hypothyroid patients before and after thyroxine replacement therapy. (Reference ranges given in parentheses.) For clarity abnormal values given in italics*

Case No	Before treatment				After treatment			
	Aspartate aminotransferase (10-35 U/l)	Alkaline phosphatase (40-100 U/l)	γ-glutamyltransferase (5-35 U/l)	Glutathione S-transferase (<4·0 μg/l)	Aspartate aminotransferase (10-35 U/l)	Alkaline phosphatase (40-100 U/l)	γ-glutamyltransferase (5-35 U/l)	Glutathione S-transferase (<4·0 μg/l)
				^{131}I treated hyperthyroid patients				
1	*47*	*213*	*63*	*10·2*	33	99	16	4·0
2	*39*	*270*	*101*	*6·8*	*44*	*248*	30	2·4
3	*40*	*306*	*217*	*4·4*	*36*	*377*	*86*	1·3
4	30	*172*	29	3·5	*38*	*133*	15	1·6
5	24	91	10	2·4	18	*132*	9	1·8
				Carbimazole treated hyperthyroid patients				
6	*44*	42	*37*	*6·2*	23	51	12	3·7
7	23	*117*	23	*9·8*	18	*127*	9	3·8
8	27	*111*	18	*8·4*	19	82	6	3·9
9	29	73	10	*10·0*	23	88	8	4·0
10	25	60	10	*10·0*	18	71	10	*7·0*
11	32	*110*	*36*	*11·0*	21	*118*	12	*8·8*
12	21	89	9	*9·4*	21	50	15	*6·3*
13	24	73	16	*5·2*	24	87	10	3·8
14	24	61	13	*9·3*	28	89	9	*14·0*
				Thyroxine treated hypothyroid patients				
15	16	40	5	1·0	28	46	6	1·0
16	20	65	15	1·2	23	80	17	2·3
17*	23	56	17	1·7	23	59	*41*	*7·9*
18*	35	95	*41*	2·6	*43*	94	*94*	*15·6*
19	17	44	9	1·5	21	70	8	3·0
20	33	99	*<5*	2·4	*72*	*125*	25	*11·4*
21	28	*106*	16	1·7	35	*109*	14	2·9
22*	26	53	*<5*	2·3	21	77	8	*7·7*

*Patient with raised free T4 concentration after replacement therapy.

(fig 1). In one patient (case 1) the glutathione S-transferase value was at the upper limit of the reference range after treatment, but in this patient the ^{131}I had produced hypothyroidism necessitating thyroxine replacement therapy.

Table I gives the results of the standard liver function tests. The three patients in whom glutathione S-transferase was raised also showed abnormalities in aspartate aminotransferase, alkaline phosphatase, and γ-glutamyltransferase activities. In addition, one further patient had an isolated increase in alkaline phosphatase activity. Patients did not show a significant fall in aspartate aminotransferase or alkaline phosphatase after treatment, but γ-glutamyltransferase activities did fall significantly ($p < 0.05$) with treatment.

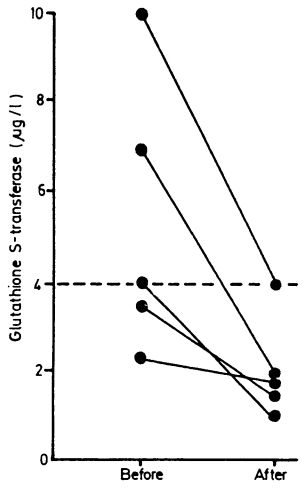

FIG 1—Plasma glutathione S-transferase measurements in five patients before and after treatment with ^{131}I for hyperthyroidism. Dashed line represents upper limit of reference range.

HYPERTHYROID PATIENTS GIVEN CARBIMAZOLE

All patients treated with carbimazole initially had raised plasma glutathione S-transferase values. After treatment all showed a significant ($p < 0.01$) fall in values. Although the fall in plasma glutathione S-transferase tended to parallel the fall in total and free T4 concentrations, equivocal or raised glutathione S-transferase values were still found up to five months after the start of treatment (fig 2). No significant difference in glutathione S-transferase values after treatment were observed between the different carbimazole treatment regimens.

In several patients there was a transient rise in glutathione S-transferase after the initiation of treatment (fig 2). In one (case 14), who showed a sensitivity reaction of a skin rash and arthralgia, the rise was pronounced. In this patient subsequent treatment with propylthiouracil produced a similar rise in glutathione S-transferase with a concomitant sensitivity reaction (fig 3). In this patient treatment was subsequently changed to ^{131}I.

Although glutathione S-transferase values were raised in all patients before treatment, only four showed abnormalities in standard liver function values (table I). After treatment with carbimazole there was a significant ($p < 0.05$) fall in activities of aspartate aminotransferase and γ-glutamyltransferase, normal values being found in all treated patients.

PATIENTS WITH HYPOTHYROIDISM

Each of the eight patients with hypothyroidism initially had normal plasma glutathione S-transferase values (fig 4) and all had raised concentrations of thyroid stimulating hormone and low concentrations of total and free T4. The concentration of total or free T3 was

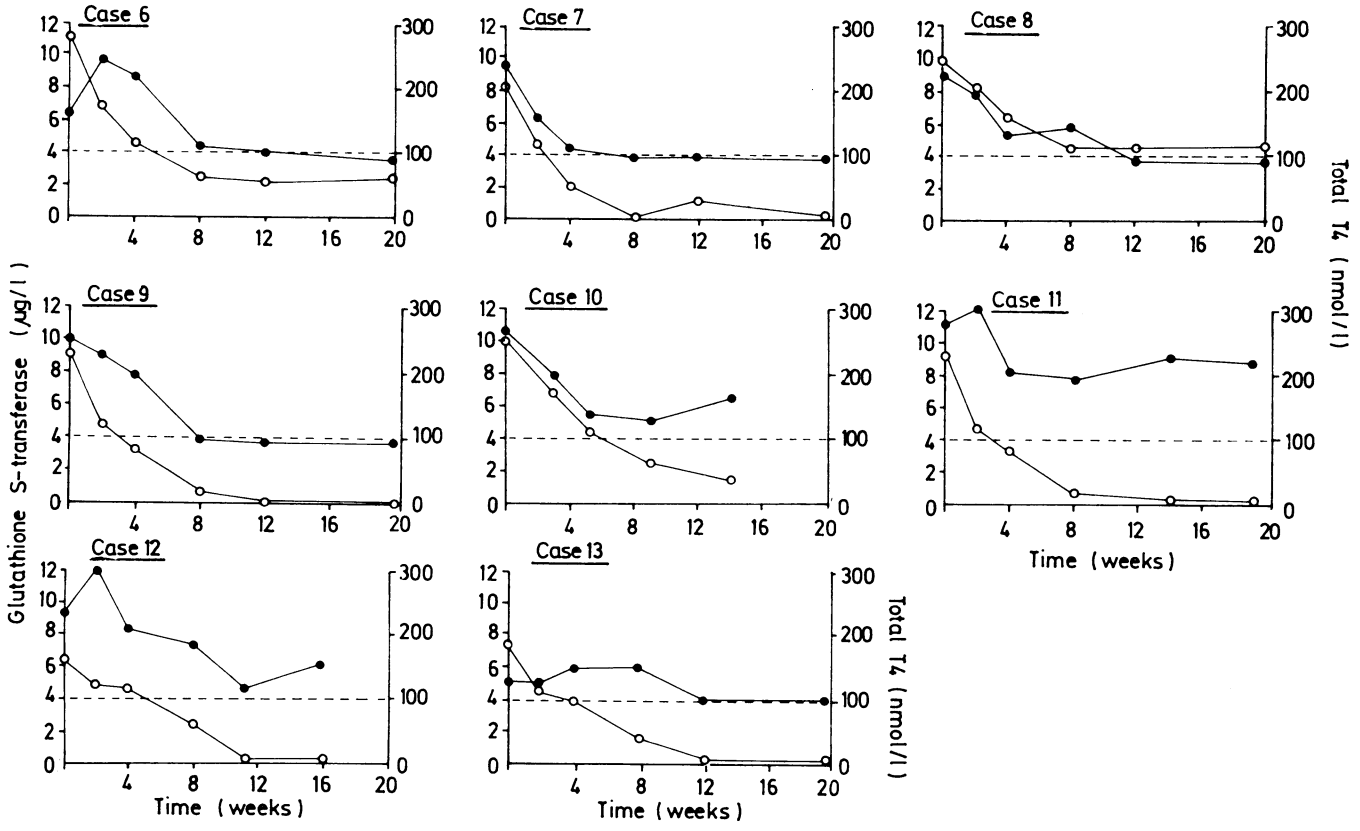

FIG 2—Sequential plasma measurements of total T4 (○) and glutathione S-transferase (●) in eight patients treated for hyperthyroidism with carbimazole. Three patients (cases 6-8) had dose of carbimazole reduced from 15 mg thrice daily in light of clinical and biochemical response. Remaining patients received continual blocking dose of carbimazole (15 mg thrice daily) and began triiodothyronine replacement (20 μg) after one month. Dashed lines represent upper limit of reference range for glutathione S-transferase. Reference range for total T4, 65-145 nmol/l.

Conversion: SI to traditional units—Total T4: 1 nmol/l ≈ 0·08 μg/100 ml.

TABLE II—*Thyroid hormone concentrations before and after thyroxine replacement therapy in eight consecutive patients with primary hypothyroidism. (Reference ranges given in parentheses)*

Case No	Before thyroxine				After thyroxine				Thyroxine dose (μg/24 h)	Duration (months)
	T4		T3		T4		T3			
	Total (65-145 nmol/l)	Free (10-22 pmol/l)	Total (1·2-2·8 nmol/l)	Free (4·0-7·8 pmol/l)	Total (65-145 nmol/l)	Free (10-22 pmol/l)	Total (1·2-2·8 nmol/l)	Free (4·0-7·8 pmol/l)		
15	43	5	1·9	5·2	112	21	1·4	5·9	100	8
16	55	7	1·6	4·2	108	17	1·3	4·1	100	8
17*	<25	2	0·7	<1·0	176	35	1·9	7·1	200	9
18*	27	4	1·3	3·5	136	28	1·9	7·1	150	3
19	65	9	1·5	4·0	121	19	1·3	4·2	150	8
20*	<20	4	1·1	3·7	91	16	1·8	5·3	100	5
21	31	4	1·6	3·2	109	17	1·7	5·4	150	8
22*	<20	<1	<0·5	1·9	116	31	1·8	7·7	200	6

*Patients with abnormal glutathione S-transferase value after replacement therapy.
Conversion: SI to traditional units—Total T4: 1 nmol/l ≈ 0·08 μg/100 ml. Free T4: 1 pmol/l ≈ 0·8 pg/ml. Total T3: 1 nmol/l ≈ 0·7 ng/ml. Free T3: 1 pmol/l ≈ 0·7 pg/ml.

subnormal in five of these patients (table II). While three patients showed clearly abnormal free T4 concentrations after treatment, only one showed an abnormal total T4 value (table II).

After thyroxine replacement therapy all patients showed a significant ($p < 0·01$) rise in plasma glutathione S-transferase (fig 4). In four patients clearly raised values were found, similar to those observed in the hyperthyroid patients. During thyroxine replacement three patients had raised free T4 concentrations, and in each of these

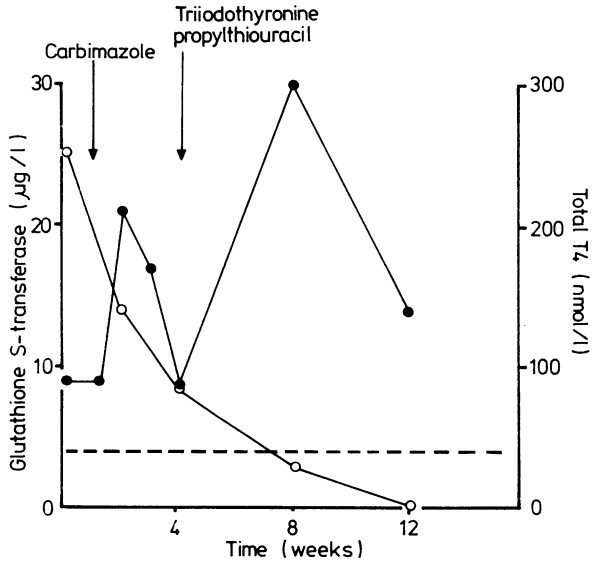

FIG 3—Case 14. Sequential total T4 (○) and glutathione S-transferase (●) measurements in patient treated for hyperthyroidism with carbimazole and subsequently with propylthiouracil and supported with triiodothyronine. After treatment with carbimazole and propylthiouracil (arrowed) pronounced sensitivity reaction occurred. Dashed line represents upper limit of reference range for glutathione S-transferase.

raised glutathione S-transferase values were found. The patient in whom free T4 was normal and glutathione S-transferase raised had been receiving long term treatment with mefenamic acid. This patient also showed abnormalities in aspartate aminotransferase and alkaline phosphatase activities after treatment with thyroxine. In all other patients normal free T4, total T4, and glutathione S-transferase values were found after treatment. All eight patients had normal concentrations of free and total T3 and either normal or suppressed concentrations of thyroid stimulating hormone. There appeared to be no association between glutathione S-transferase values after treatment and the dose or duration of thyroxine replacement therapy.

After thyroxine replacement therapy there was a significant ($p < 0·05$) increase in alkaline phosphatase activity (table I). Two of the patients with raised free T4 concentrations after treatment had abnormalities in γ-glutamyltransferase, and in one of these patients aspartate aminotransferase activity was also increased.

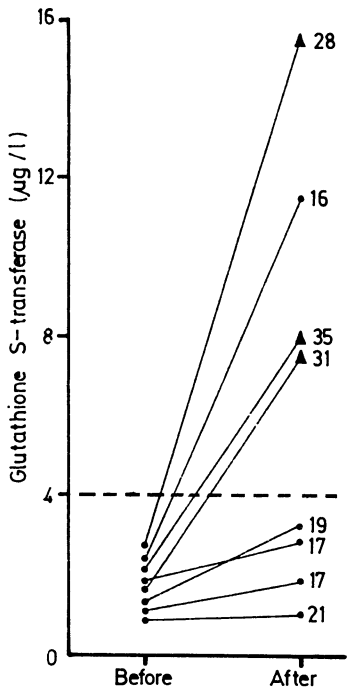

FIG 4—Plasma glutathione S-transferase measurements in hypothyroid patients before and after thyroxine replacement therapy. ▲ = Patients with raised free T4 concentrations after treatment (actual values shown in pmol/l). All patients had normal total and free T3 values after treatment.
Conversion: SI to traditional units—Free T4: 1 pmol/l ≈ 0·8 pg/ml.

Discussion

It is generally thought that hepatic dysfunction is no longer a common complication of hyperthyroidism, since abnormalities in results of the standard chemical tests of liver function are found infrequently. We, however, have shown that a high proportion of patients with untreated hyperthyroidism have greatly increased plasma glutathione S-transferase values and that successful treatment with ^{131}I or carbimazole results in a fall in these values which roughly parallels the fall in total T4 concentration.

Unlike aspartate aminotransferase, plasma glutathione S-transferase values appear to correlate well with the histological picture in patients with chronic active liver disease,[7] and also unlike alanine aminotransferase the incidence of abnormal glutathione S-transferase values in patients with paracetamol poisoning[8] agrees well with the incidence of abnormal histological findings in the livers of these patients.[13] In this context

our finding of a high incidence of raised plasma glutathione S-transferase values in hyperthyroidism agrees with the previously reported observation, with electron microscopy, that minor structural abnormalities commonly occur in the organelles of the hepatocytes of patients with this disease.[2] Our observations are therefore consistent with the hypothesis that minor hepatocellular dysfunction may still be a common though subclinical complication in hyperthyroidism.

Glutathione S-transferases are a "multigene" group of enzymes that are concerned in the cellular detoxification of a wide range of electrophiles. In human liver and kidney the main forms of the enzymes are cationic (pI 8·4-8·9), while in other tissues an anionic form (pI 4·8) predominates. These enzymes are dimeric, and in human liver two cationic subunits, B_1 and B_2, have been identified. The radioimmunoassays which we use are specific for either the B_1 or B_2 monomers and do not react with the anionic forms of glutathione S-transferase. As well as the plasma values of hepatic glutathione S-transferase B_1B_1 we also measured hepatic glutathione S-transferase B_2B_2 (previously called N/A2b).[11 12] For clarity these data have not been included but in all cases abnormal basic glutathione S-transferase B_1B_1 values were associated with abnormal values of glutathione S-transferase B_2B_2. The subunit forms of glutathione S-transferase which we have measured specifically by radioimmunoassay are confined predominantly to the hepatocyte and the renal tubules, little of these subunit forms being found in tissues such as muscle, red cells, or lung.[14]

We have considered the possibilities that the raised plasma glutathione S-transferase values found in the hyperthyroid patients were a consequence of hepatic enzyme induction by thyroxine or may merely have reflected extrahepatic tissue damage. Both these possibilities seem unlikely. Firstly, when thyroxine is administered to hypothyroid or euthyroid animals either a small decrease or no change in hepatic glutathione S-transferase is observed.[15] Secondly, as the cationic forms of glutathione S-transferase are relatively specific to the liver and kidney, and since renal damage is not found in hyperthyroidism, it appears that the abnormal plasma values of both B_1 and B_2 originate from hepatic damage.

The cause of the hepatic lesions observed in hyperthyroidism is not clear but is believed to be multifactorial, with selective and general hypoxia as contributory factors.[1] Thyroxine itself has not been reported as directly hepatotoxic. Nevertheless, our observation that prolonged administration of thyroxine to hypothyroid patients resulted in significant increases in plasma glutathione S-transferase in all patients has implications for the long term management of these patients. It is also relevant to the controversy about the importance of raised plasma free T4 concentrations in patients receiving thyroxine.

In our study all of the treated hypothyroid patients with raised free T4 concentrations had associated abnormalities in plasma glutathione S-transferase values. Our data may indicate that administration of thyroxine in doses which result in raised plasma concentrations of free T4 may produce a hepatic lesion similar to that seen in hyperthyroidism. Jennings *et al* have shown that hypothyroid patients who are receiving thyroxine replacement and in whom free and total T4 concentrations are raised have a decreased systolic time interval, which suggests that they have a "tissue thyrotoxicosis."[5] Our data suggest that they may also have a "liver thyrotoxicosis."

The thyroid gland secretes approximately 90 μg thyroxine a day into the peripheral circulation. Patients who are receiving oral replacement therapy will receive roughly this amount as a single dose. Administered in this way, high concentrations of thyroxine are presented to the liver, through the portal vein. Thus, although peripheral blood concentrations of thyroid hormone may appear satisfactory, it is possible that in these patients liver thyrotoxicosis results from an exposure to high portal concentrations of thyroxine.

Plasma glutathione S-transferase values returned to normal in hyperthyroid patients treated with ^{131}I, but in patients treated with carbimazole they remained raised even five months after treatment began. In roughly half of the carbimazole treated patients glutathione S-transferase values were increased in the first sample after treatment; in one patient this rise was pronounced and associated with physical signs of a sensitivity reaction. It appears that carbimazole may cause transient subclinical liver damage in some patients, and hepatobiliary problems associated with carbimazole, methimazole, and propylthiouracil have been reported in a few.[16-18] Our data, however, do not show that carbimazole is necessarily hazardous, since the rises in plasma glutathione S-transferase after carbimazole were transient and patients are unlikely to receive carbimazole for long periods. On the other hand, our finding that hepatic abnormalities may occur in patients receiving thyroxine replacement is of concern, since for these patients treatment will be life long. Hepatic function should therefore be considered and assessed periodically in such patients. Measurement of the plasma glutathione S-transferase value by radioimmunoassay appears to provide the most sensitive and specific method of assessing hepatic function in these patients. Nevertheless, the availability of glutathione S-transferase assays is restricted to a few centres, and it is therefore reassuring to note that three of the four hypothyroid patients who had raised glutathione S-transferase values after treatment would have been detected by the standard liver function tests. These tests may offer a satisfactory alternative to glutathione S-transferase measurements in clinical practice.

In conclusion, our data, and those of others,[5] indicate that the thyroxine dose should be lowered in patients who have raised free thyroid hormone concentrations in order that free T3 and free T4 may revert to normal.

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