Thyroid hormone and carrier protein interrelationships in children recovering from kwashiorkor[1-3]

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ABSTRACT We have studied 15 infants with severe protein energy malnutrition (PEM) as a model of nutritional nonthyroidal illness. Changes in circulating thyroid hormones, binding proteins, and their interrelationships were assessed before and during recovery. Serum concentrations of total thyroxine and triiodothyronine and of thyroxine-binding proteins were extremely reduced, and increased progressively during 3 wk of refeeding. The T_4:TBG molar ratio was initially 0.180 ± 0.020, and increased progressively, parallel to the increases in TT_4, to 0.344 ± 0.038 after 21 days ($p < 0.025$). The changes in free T_4 estimates varied according to the methods used—FTI and analogue FT_4 increased, dialysis FT_4 fraction decreased. Serum TSH levels increased transiently during recovery.

It is concluded *1*) there is reduced binding of T_4 and T_3 to TBG in untreated PEM which takes 2–3 wk to recover; *2*) there are methodological differences in evaluating free T_4 levels in PEM; *3*) increased TSH secretion appears to be an integral part of the recovery from PEM. *Am J Clin Nutr* 1986;43:406–413.

KEY WORDS Protein energy malnutrition, thyroxine, triiodothyronine, reverse triiodothyronine, thyroxine-binding globulin, prealbumen, thyrotropin

Introduction

A variety of changes in circulating thyroid hormones may occur in nonthyroidal illnesses (NTI) (1–3). Low serum levels of triiodothyronine (T_3) are found most often (1, 2), and are attributed to the reduction of peripheral deiodination of thyroxine (T_4) to T_3, often accompanied by an accumulation of reverse T_3 (rT_3) with resultant relative or absolute elevations of this metabolite (2). The serum-free fraction of T_4, which is in equilibrium with the protein-bound hormone, is characteristically elevated in NTI (2, 3). Free T_4 levels (FT_4) have been said to be influenced by alterations in concentrations of thyroxine-binding proteins—albumen (4), thyroxine-binding prealbumen (TBPA) (5, 6), and thyroxine-binding globulin (TBG) (7), in addition to inhibitors of T_4 binding to carrier proteins. Albumen and especially TBPA (8) are sensitive to nutritional status.

Most studies of NTI have involved groups of patients with established diseases and have not evaluated serial changes in thyroid function tests as the patients' condition improves. In infantile protein energy malnutrition (PEM) concentrations of all three thyroid hormone-binding proteins are extremely low (8–11) and recover rapidly with refeeding. This condition thus provides a nutritional model of NTI in which thyroid hormones, carrier proteins, and their interactions can be studied at various protein concentrations as recovery supervenes. We have therefore investigated prospectively

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[2] Supported by a grant from the South African Medical Research Council. REM was a recipient of the Wellcome-Lowenstein Research Travel Grant.

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Received May 28, 1985.

Accepted for publication September 24, 1985.

changes in circulating thyroid hormone levels and the relation of T_4 and T_3 to carrier protein concentrations in children with PEM, before and during progressive recovery to normality. Various methods for expressing free T_4, direct and derived, have also been evaluated.

Patients and methods

Fifteen children with PEM, ages 7 to 27 mo (mean, 12.5 mo) were studied. All presented with edema and the typical clinical evidence of severe kwashiorkor (Wellcome Working Party classification) (12). All but one child presented with a history of vomiting associated with diarrhea in most, with respiratory infections in three and impetigo in three others. All were edematous. Only three did not require initial intravenous therapy, which generally lasted 3 to 4 days, especially if parenteral antibiotics were used. All children were given antibiotics (ampicillin, or penicillin and gentamycin) and vitamin supplements. Intercurrent infections and enteritis were usually better within the first week. Only enteral refeeding was used, starting within the first 24 h after admission, and was continued until full clinical recovery had been achieved, after 14 to 21 days. One patient received a blood transfusion. Tests of renal and liver function are shown in **Table 1.** Serum urea and creatinine levels were initially low; urea rose with refeeding. Liver function tests were normal.

Blood was withdrawn on the day of admission to hospital and weekly thereafter for 2 (all subjects) or 3 wk (six subjects). The separated serum was stored at −20°C for up to 6 mo before all assays were completed. Sera from the first 10 subjects studied were assayed for albumen, TBG (13), total T_4 (TT_4), T_3, rT_3, the T_3 resin uptake test (T_3RU), free T_4 by a direct analogue method (AFT_4) (Amerlex FT_4, Amersham, UK), and thyrotropin (TSH) (14); the free thyroxine index (FTI) was calculated $T_4 \times T_3RU \div 100$. In six subjects FT_4 was measured by equilibrium dialysis (DFT_4) (15) and TBPA was assayed by immunoelectrophoresis (rocket electrophoresis) (16) in addition to the T_4 measurements. All sera from each subject were assayed together to avoid interassay variability.

Electrophoretic studies. 50 ul of serum was incubated with 50 ul ^{125}I–T_4 in phosphate buffered saline (20,000 cpm) for 30 min at 37°C prior to cellulose acetate electrophoresis in 0.05 M barbitone buffer, pH 8.6, with sodium azide as preservative. After autoradiography the TBG, albumen, and TBPA areas were cut out and counted. Sera from six patients, from before and after recovery were evaluated in this manner; each pair was studied in duplicate in the same electrophoretic run. (One subject was studied during and after recovery.)

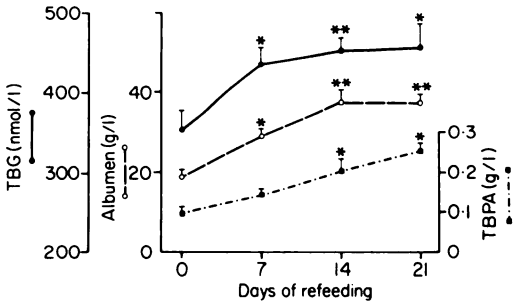

FIG 1. Changes during refeeding of mean (±SEM) serum concentrations of thyroxine-binding globulin (TBG), albumen, and thyroxine-binding prealbumen (TBPA) in children with protein energy malnutrition. * = compared to day 0, $p < 0.05$. ** = compared to day 0, $p < 0.01$.

The committee for Research on Human Subjects of the University of the Witwatersrand approved the study.

Statistical analyses. The paired *t* test was used to evaluate changes with time. Correlations were assessed by linear regression analysis, and the slopes and intercepts of regression lines were compared by the analysis of covariance. Data are expressed as the mean ± SEM.

Results

On admission to the hospital the levels of albumen, TBPA, and TBG were subnormal in every subject but had risen to within the normal ranges by the day 14 (**Fig 1**) (normal ranges: albumen, 34–46 g/l; TBPA, 0.24–0.40 g/l; TBG, 344–718 mmol/l).

The alterations in TT_4, T_3, rT_3, and TSH after refeeding are shown in **Figure 2** (normal ranges: TT_4, 60–150 nmol/l; T_3, 1.5–3.0 nmol/l; rT_3, 0.10–0.37 nmol/l; TSH, <8 μU/ml). At presentation T_3 levels were subnormal in every case, and TT_4 levels in 10 of the 15 children; by day 14, levels were normal in every patient. Reverse T_3 levels were elevated in

TABLE 1
Tests of kidney and liver function in PEM children before and 21 days after refeeding

	Initial	Final	Reference range
Urea (mmol/l)	1.8 ± 0.3	4.0 ± 0.5 ($p < 0.01$)	<6.0
Creatinine (μmol/l)	27.3 ± 3.3	29.9 ± 2.0	30–60
Alkaline Phosphase (μ/l)	84.7 ± 5.6	101.1 ± 12.2	150–380
ALT (μ/l)	11.9 ± 0.6	17.3 ± 2.9	<35
AST (μ/l)	7.3 ± 1.1	14.3 ± 2.9	<40

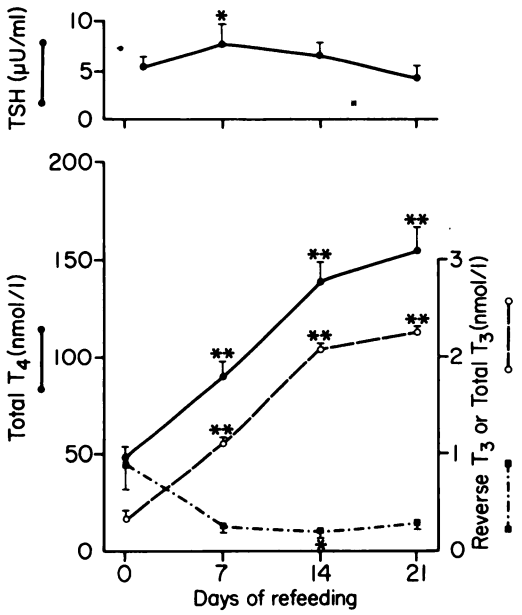

FIG 2. Alterations in mean (±SEM) serum concentrations of thyrotropin (TSH), total thyroxine (TT_4), total triiodothyronine (TT_3), and reverse T_3 (rT_3) in children with protein energy malnutrition, before and during 21 days refeeding. * = compared to day 0, $p < 0.05$. ** = compared to day 0, $p < 0.01$. O = compared to TSH level on day 7, $p < 0.05$.

11 children and declined rapidly within 7 days of refeeding and remained fairly constant thereafter. In patients with normal rT_3 levels on admission initial TT_4 concentrations were extremely low (12–29 nmol/l). Mean serum TSH levels increased from 5.1 ± 0.9 μU/ml (range, 2.2–11.9 μU/ml) to peak levels on either day 7 or day 14 (two subjects). The mean value of the peak levels was 8.2 ± 1.8 μU/ml (range, 2.2–18.4 μU/ml; $p < 0.05$) (increments ranged from −0.5 to +12.3 μU/ml); and then declined in every case by day 21, to 3.0 ± 1.5 ml/ml (vs peak levels $p < 0.05$); at this stage TSH levels were below initial values in four of the six children studied.

The differences in the rates of relative changes (percent of recovery values) of TT_4, TBG, and the T_4:TBG molar ratio are shown in **Figure 3.** Only after 7 days did the T_4:TBG ratio increase, parallel with the rise in T_4. TBG levels were very close to normal during this period.

The various assessments of Free Thyroxine are shown in **Table 2.** Mean values of the T_3RU test declined progressively from baseline until day 14. Despite this fall FTI values increased progressively from day 1 to day 21. The T_4:TBG molar ratio was very low on day 1 and did not change significantly in the first week. Thereafter values rose progressively, and significantly by days 14 and 21. Levels of FT_4 measured by the analogue method, (AFT_4) also rose progressively with recovery. In contrast the free fraction of thyroxine, assessed by equilibrium dialysis, declined progressively. However, the mean-derived dialysis FT_4 (DFT_4) remained virtually constant for the 21 days of the study.

Serum T_3 levels were undetectable (<0.1 nmol/l) in half the children on admission to hospital, but measurable levels were found 1 wk later in all. The T_3:TBG molar ratio increased progressively with refeeding from 0.0027 ± 0.0006 on day 7 to 0.0045 ± 0.0005 on day 14 ($p < 0.025$) to 0.0053 ± 0.0006 on day 21 ($p < 0.05$). The molar T_3:T_4 ratio was extremely low on day 1, and increased substantially (200% of basal) by day 7 and remained constant thereafter (**Fig 4**). There was a reciprocal fall in the rT_3:T_4 ratio between days 1 and 7, values remaining almost constant over the subsequent 2 wk.

Correlations. Total T_4 was significantly cor-

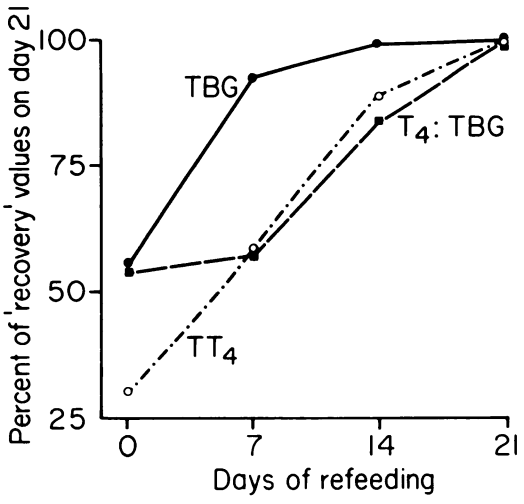

FIG 3. Relative changes in thyroxine-binding globulin (TBG), and total thyroxine (TT_4) and the T_4:TBG molar ratio in children with protein calorie malnutrition, before and during refeeding. Recovery values on day 21 were designated 100%.

TABLE 2
Changes in total T_4 levels, T_3RU values and the various methods of expressing Free Thyroxine before and during refeeding in children with PEM (only six children were studied on day 21). (AFT_4 = FT_4 by analogue method; DFT_4 = FT_4 by equilibrium dialysis)

Days of refeeding	0	7	14	21	Normal range
TT_4 (nmol/l)	46.9 ± 5.3	89.0 ± 8.0**	138.8 ± 10.5**	154.7 ± 10.8**	60–150
T_3RU (%)	40.4 ± 2.5	32.8 ± 1.6**	28.5 ± 3.2**	29.8 ± 1.9*	30–40
FTI	15.5 ± 2.5	29.7 ± 2.7*	38.7 ± 2.5**	44.2 ± 1.9*	25–60
AFT_4 (pmol/l)	7.6 ± 1.4	10.9 ± 1.2	14.2 ± 3.8*	22.7 ± 4.1*	12–25
DFT_4 (%)	0.037 ± 0.007	0.024 ± 0.007	0.020 ± 0.005	0.017 ± 0.002*	0.018–0.040
DFT_4 (pmol/l)	19.5 ± 3.9	19.1 ± 4.2	18.1 ± 4.9	20.8 ± 4.6	16.9–38.4
T_4:TBG	0.180 ± 0.020	0.192 ± 0.018	0.281 ± 0.025**	0.344 ± 0.038*	—

* vs day 0, $p < 0.05$.
** vs day 0, $p < 0.01$.

related with TBG levels on days 0, 14, and 21 of the study ($p < 0.05$–$p < 0.001$) (**Figure 5**). The slopes of the regression lines were all comparable, but the y intercept on day 1 was significantly ($p < 0.01$) lower than on day 21. There were no correlations between T_4 or T_3 and albumen or TBPA, and T_3 levels did not correlate with TBG.

Analogue FT_4 values did not correlate with any of the carrier protein concentrations, nor with the T_4:TBG molar ratio or the FTI on each day of the study. FTI values correlated with T_4:TBG molar ratios on day 7 ($r = 0.87$, $p < 0.02$) and on day 14 ($r = 0.65$) ($p < 0.05$). Dialysis FT_4 fraction correlated negatively with the T_4:TBG molar ratio ($r = -0.71$, $p < 0.01$), but not with TBG, TBPA, or albumen levels.

Changes in T_3 or T_4 levels and TSH did not correlate.

*Electrophoretic studies (**Table 3**).* In this small group of children the changes in levels of TT_4- and T_4-binding proteins before and after recovery were similar to the group as a whole. The bound T_4:TBG molar ratio increased by 36% ($p < 0.05$) during recovery from PEM, reflecting the changes in the serum T_4:TBG ratio. In contrast, the bound T_4:TBPA ratio was 55% greater before refeeding compared to recovery values ($p < 0.05$). The changes in albumen-bound T_4 were inconsistent.

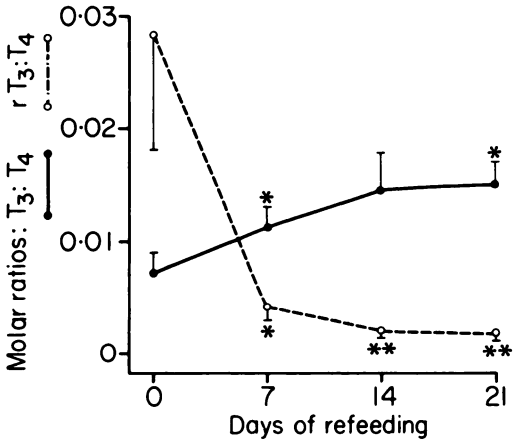

FIG 4. The molar ratios of total triiodothyronine:thyroxine and reverse triiodothyronine:thyroxine in children with protein energy malnutrition before and during refeeding. * = compared to day 0, $p < 0.05$. ** = compared to day 0, $p < 0.01$.

Discussion

The circulating concentrations of thyroid hormones and their binding proteins in our children with PEM were similar to previously published values (8, 10, 11). With refeeding and recovery all levels normalized rapidly. Serum TBG concentrations after recovery were comparable to those seen in West African children and were higher than reported in Caucasian children (10, 11). During refeeding TBG levels recovered most rapidly, while the recovery of albumen concentrations appeared to be faster than those of TBPA.

Most circulating T_4 is normally bound to TBG (17). However, in untreated children with PEM the very low serum concentrations of T_4 cannot be explained only by the reduced levels of TBG and the other T_4-binding proteins. After 3 wk of refeeding the mean TBG concentration had increased by about 65% of

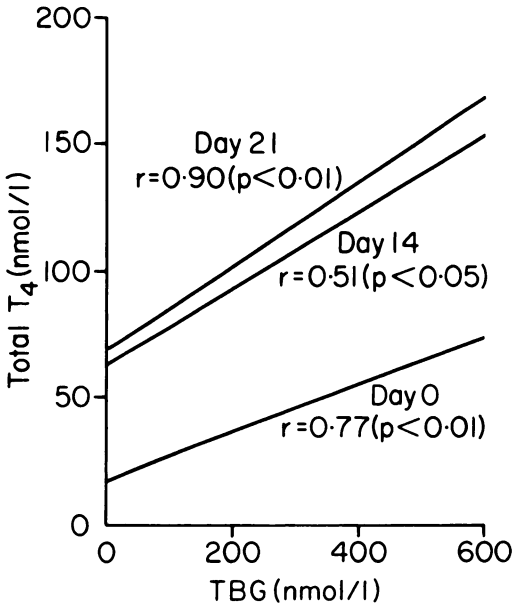

FIG 5. The linear regression lines of total thyroxine (TT_4) against thyroxine-binding globulin (TBG) concentrations in children with PEM before therapy and on days 14 and 21. The y intercept (TT_4) was significantly greater on day 21 compared to day 0 ($p < 0.01$). Day 0: $y = 0.095x + 17.7$; day 21: $y = 0.17x + 69.1$.

pretreatment values, but T_4 levels rose by more than 200%. Moreover, in each individual the T_4:TBG molar ratios were initially extremely low and increased substantially in every case only after 7 days of treatment. Thus the degree of saturation of TBG with T_4 remained virtually unchanged during the first week of refeeding, and then increased progressively from day 7 to full recovery on day 14 or 21, in parallel with the increases in TT_4. The parallelism of the T_4:TBG correlations on days 0 and 21, with an increase in the T_4 intercept also demonstrates the overall increase in TBG saturation after recovery.

In the untreated patients reduced thyroid hormone production may have contributed to the extremely low level of TBG saturation with T_4: the T_4-degradation rate, and by implication its production rate, is reduced in the steady state of untreated PEM (11). After 7 days of optimal refeeding T_4 kinetics have largely normalized (12), yet in our patients TBG saturation remained unchanged, despite increases in both T_4 and TBG. These findings suggest that there is interference with T_4 binding to TBG in PEM. This concept is also supported by the significant rise in the T_4 intercept of the T_4:TBG correlations between days 0 and 21, and by the electrophoretic studies. This pattern of decreased T_4 binding has been observed in patients with NTI, with either normal or elevated TBG concentrations (13). Thus the effects of NTI contribute substantially to the very low serum concentrations of T_4 found in PEM. The change from high to low normal values of the T_3RU test, which reflects the number of available thyroid hormone-binding sites on carrier proteins (18), also supports this concept.

A similar pattern of recovery in the T_3:TBG molar ratio was also evident from days 7 to 21 of refeeding. Most circulating T_3 is derived

TABLE 3
Concentrations of TT_4, binding proteins, and percentage ^{125}I-T_4 bound to each protein after electrophoresis of serum, before and during refeeding in six PEM children

		TBG			TBPA			Albumen		
	Serum TT_4	Concentrations	T_4-bound	T_4-bound/TBG	Concentrations	T_4-bound	T_4-bound/TBPA	Concentrations	T_4 bound	T_4-bound/albumen
	nmol/l	*nmol/l*	%		*nmol/l*	%		*g/l*	%	
Before treatment	62.2 (8.9)*	524.7 (110.6)	63.2 (3.5)	0.095 (0.022)	1186.7 (210.3)	16.8 (2.4)	0.0070 (0.0029)	24.1 (1.9)	20.0 (1.8)	0.57 (0.06)
During recovery	112.3 (15.2)	682.9 (56.9)	69.9 (2.1)	0.130 (0.021)	3277.1 (574.7)	13.3 (1.7)	0.0045 (0.0026)	38.6 (1.5)	16.8 (1.5)	0.53 (0.08)
Significance	$p < 0.025$	$p < 0.025$	$p < 0.05$	$p < 0.025$	$p < 0.05$	NS†	$p < 0.05$	$p < 0.01$	$p < 0.1$	NS

The bound T_4:protein ratio is a measure of the degree of saturation of the protein with T_4.
* SEM in parenthesis.
† NS = not significant.

from the peripheral conversion of T_4, by 5'deiodinase (19), an enzyme system which also rapidly metabolizes rT_3 (20). Thus the relatively elevated serum levels of rT_3, and the very low levels of T_3 reflect inhibition of the 5'deiodinase systems, characteristic of NTI (21). In PEM, the rapid decline of rT_3 to very low levels suggests that the 5'deiodinase system recovers within a week of refeeding. The reciprocal rise in T_3 levels, however, takes longer to normalize. The progressive increase in T_3:TBG ratio suggests that binding of T_3 to TBG is also initially inhibited in PEM, and takes time to recover. Additionally, the unchanged T_3:T_4 molar ratio from day 7 to 21 suggests that the availability of T_4 might be a limiting factor in the full recovery of serum T_3 levels. Lack of T_4 may also have contributed to the relatively low levels of rT_3 seen in a few subjects. This concept is supported by the studies of isolated perfused rat livers in which hepatic T_3 production is dependent on the T_4 concentrations in the perfusate (22). Moreover, in NTI the T_4-protein binding inhibitory factors may also reduce the accumulation of cellular T_4 (23).

The initial mean serum TSH level was somewhat raised compared to the mean recovery value (24), and concentrations increased significantly with early refeeding, and then fell to lower levels with complete recovery. Prolonged fasting in normal subjects lowers basal and TRH-stimulated TSH levels (25): thus the rise in TSH with refeeding may represent recovery from the effects of starvation. Recent improvement in nutritional status may account for the occasional elevated TSH level seen in patients with NTI. An alternative explanation, that the pituitary sensitivity to ambient thyroid hormone levels may normalize with improved nutritional status, leading to initial increases in TSH secretion in the presence of subnormal T_4 concentrations, appears unlikely. The pituitary retains sensitivity to rising T_3 levels and to TRH in kwashiorkor (24). Moreover, the thyroidal radioactive iodine uptake and clearance are normal in kwashiorkor (26); and thirdly the thyroid remains sensitive to TSH even after prolonged periods of severe malnutrition (27). Furthermore the absence of correlations between changes in TSH and T_3 or T_4 levels in the present and a previous report (28) also argue against this hypothesis. Our data from patients with moderately severe infective illnesses and severe malnutrition, do not support the concept that TSH levels are universally low in severe NTI (29) although the TSH assay was not ultrasensitive. After 21 days of refeeding TSH concentrations were lower than initial values in four of six cases. Nevertheless, the rise in TSH accompanying refeeding is likely to accelerate the recovery of circulating T_4 and T_3.

The free or unbound fraction of T_4 is generally considered to be the biologically available and therefore the active form of the hormone. For this reason we evaluated a variety of in vitro indices of free thyroxine during recovery from PEM. The FTI, T_4:TBG ratio, and analogue FT_4 measurements were all extremely low at presentation and recovered progressively during refeeding. In contrast, the FT_4 fraction, assessed by equilibrium dialysis, progressively declined with refeeding; this fall was fully compensated for by the reciprocal rise in TT_4, resulting in unchanged levels of DFT_4, confirming an earlier report (11). The normal DFT_4 in childhood PEM contrasts with the elevated levels observed in adult malnutrition and other NTIs, where both the free fraction and absolute FT_4 are often elevated (30). The reasons for this discrepancy are not clear.

Very reduced concentrations of FT_4 measured by the direct analogue method have recently been reported in PEM (31) comparable to levels we observed using a similar method. Subnormal FT_4 values assayed by these methods can be caused by reduced levels of albumen (4) or TBPA (6) and by elevated levels of free fatty acids (32). We could not confirm an association with either of these proteins, nor with TBG, before, during, or after recovery from PEM, in individual children or in the group as a whole. Nor did AFT_4 correlate with DFT_4 or FTI, nor with the T_4:TBG ratio, although these measurements correlate well in normal subjects (33). Precisely what AFT_4 levels reflect in patients with NTI is unclear (34, 35). On the other hand the dialysis FT_4 fraction, but not the absolute level, did correlate, inversely, with the T_4:TBG ratio.

The reasons for these differences in apparent free T_4 levels may be explained by the principles of the assay methods. The radio-labeled T_4 analogue used in the assay system we used

binds to serum proteins (6). Any substance which displaces T_4 from proteins should also reduce the proportion of protein-bound analogue, resulting in an increased proportion of unbound analogue. Thus the assay antibody will take up more label giving a spuriously low FT_4 estimation. In contrast, reduced T_4 binding is measured directly as an increase in the free T_4 fraction in the equilibrium dialysis system, and is reflected by a reduced T_4:TBG ratio.

The electrophoretic studies confirm a reduction of T_4 binding by TBG in untreated compared to recovering PEM. Moreover, there appeared to be a reciprocal *increase* in relative T_4 binding to TBPA, but not to albumen, in untreated cases, suggesting that TBPA, although reduced in concentration, buffers some of the excess unbound T_4 displaced from TBG. A similar observation has been made in uremic animals (14).

The nature of the interference with the T_4–TBG interaction and T_4 to T_3 deiodination in NTI has not been fully elucidated. Recent evidence, however, strongly suggests that elevated plasma levels of free fatty acids (FFA) may displace T_4 from TBG and increase the dialysable fraction (36), and reduce measured levels of AFT_4 (32). Moreover, in vitro studies have shown that fatty acids interfere with the production of T_3 from T_4 and may also be active in vivo (37). Plasma concentrations of FFA are considerably elevated in untreated Kwashiorkor and fall rapidly during refeeding (38), perhaps contributing to the changes in T_4 binding and to the low serum T_3 levels.

Thus it is apparent that the changes in bound and free thyroid hormones in recovering PEM did not reflect only changes in binding protein concentrations. Only changes in TBG, and not those of albumen or TBPA, were reflected by the FT_4 fraction measured by equilibrium dialysis. There are also qualitative changes in thyroid hormone binding—a decrease in TBG binding with relative increases in TBPA binding, the former predominating and contributing substantially to the very low levels of T_4 and T_3 found in untreated PEM. An increase in TSH secretion appears to be characteristic of recovery from PEM and may accelerate the normalization of circulating thyroid hormone concentrations.

We thank Dr E Sochett for allowing us to study some of his patients.

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