

Thymulin (facteur thymique sérique) and zinc contents of the thymus glands of malnourished children¹⁻³

Bernard Jambon, PhD; Olivier Ziegler, MD; Bernard Maire, PhD; Marie-France Hulin, PhD;
Gérard Parent, MD; Mohamadou Fall, MD; Daniel Burnet, PhD; and Jean Duheille, PhD, MD

ABSTRACT Protein-energy malnutrition (PEM) leads to an immune deficiency, which is now well documented. Some investigators have suggested that the associated zinc deficiency is important in thymic involution and changes in cellular immunity. To evaluate the respective roles of nutritional deficiency, infection, and zinc in the alteration of thymic function, we measured the amounts of thymulin (facteur thymique sérique, or FTS) and of Zn in the thymus glands of 58 Senegalese children who died in various stages of malnutrition. In the severe forms (marasmus, kwashiorkor, and marasmic kwashiorkor) the thymus was tiny and contained very little thymulin. The Zn content of the thymus was high whatever the nutritional state of the subject and was related significantly only to the presence of infections. In Senegalese children thymic atrophy and depleted thymulin content are associated with severe PEM but not systemic infection or depleted thymic Zn content. *Am J Clin Nutr* 1988;48:335-42.

KEY WORDS Protein-energy malnutrition, facteur thymique sérique, thymulin, thymus, zinc, children

Introduction

It is well established that severe forms of protein-energy malnutrition (PEM) produce a depression of cell-mediated immunity, characterized principally by a defect in maturation of peripheral T lymphocytes (1, 2). This functional disorder, which induces disturbances in distribution of subpopulations of T lymphocytes (3) and a rise in percentage of null cells (4), is thought to originate in the thymus gland. Many investigators have reported some degree of involution of the thymus both in animals killed in a state of malnutrition (5) and in children who have died from PEM (6-11). However, it is difficult, for lack of direct proof, to decide whether the lymphocyte-differentiating function of the thymus is altered.

Though a reduced lymphocyte population in the cortex of the thymus has often been reported (8, 11), very little information has been published about the functional state of the thymic epithelium (12). Recent investigations showed that thymic epithelial cells, including peripheral ones in Hassall corpuscles, are the site of production of hormones and thymic factors responsible for maturation and differentiation of T lymphocytes (13-15).

We chose to study thymulin (also known as facteur thymique sérique or FTS); the thymic origin and hormonal nature of thymulin were clearly established (16, 17). A change in the capacity of the thymic epithelium to produce the hormonal factors for lymphocyte differ-

entiation, if indeed it does occur, might be a main cause of deficiency in functional maturation of T lymphocytes observed in children with PEM.

Indirect arguments support this point of view. Isolated T lymphocytes from malnourished children, when incubated with thymic factors such as thymosin (18) or thymopoietin (19), rapidly acquire the characteristics of functional maturation that they lacked. On the other hand, the few attempts at estimating thymulin in the blood of such children gave contradictory results: whereas Chandra (20) reported a large decrease in the biological activity of thymulin in severe PEM particularly in kwashiorkor, Maire et al (21) observed no significant variation of this activity with the degree or type of malnutrition.

In this investigation we evaluate directly the amount

¹ From the Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM), Paris, France; the Département de Nutrition et Maladies Métaboliques de Nancy, Vandoeuvre les Nancy, France; the Laboratoire de Chimie, Faculté de Médecine de Nancy, Vandoeuvre les Nancy, France; the Service de Pédiatrie, Hôpital Le Dantec, Dakar, Sénégal; and the Laboratoire d'Immunologie, Faculté de Médecine de Nancy, Vandoeuvre les Nancy, France.

² Supported by la Délégation Générale de la Recherche Scientifique et Technique (DGRST) contrat 82L1189.

³ Address reprint requests to J Duheille, Laboratoire d'Immunologie, Faculté de Médecine, BP 184, F 54505 Vandoeuvre Cedex, France. Received June 30, 1986.

Accepted for publication December 16, 1986.

Cote: VII
20 SEP. 1988
85
N° : 25506 ex 1
M Cote : B

ORSTOM Fonds Documentaire

TABLE 1
Description of the sample

Nutritional-state groups	n	Age*	Sex		Infection		Weight for height*	Weight for age*	Height for age*
			Male (a)	Female (b)	Absent (a)	Present (b)			
mo									
1. Undernourished	18	11.4 ± 3.4	7	11	4	14	81 ± 2.0	78 ± 4.6	94 ± 2.0
2. Marasmus	15	8.9 ± 2.0	9	6	3	12	61 ± 1.6	52 ± 2.4	93 ± 1.6
3. Kwashiorkor	11	14.5 ± 2.2	7	4	1	10	74 ± 1.7	74 ± 2.4	99 ± 1.0
4. Marasmic kwashiorkor	14	14.6 ± 2.4	8	6	2	12	61 ± 1.3	55 ± 2.5	94 ± 1.8
Comparisons		F†	F‡	F§	F	F	F	F	F
		1/2/3/4¶	(a/b)/1/2+3+4§	(a/b)/1/2+3+4§	1/2/3/4	1/2/3/4	1/2/3/4	1/2/3/4	1/2/3/4
		1/2+3+4§			1/2+3+4	1/2+3+4	1/2+3+4	1/2+3+4	1/2+3+4
		2/3/4§			2/3/4	2/3/4	2/3/4	2/3/4	2/3/4

* $\bar{x} \pm \text{SEM}$.

† F: analysis of variance. F 1/2/3/4: comparison of the four groups; F 1/2+3+4: group 1 compared with groups 2, 3, and 4 combined; F 2/3/4: comparison of the three groups 2, 3, and 4.

‡ Frequencies, f: Fisher's exact probabilities test. f(a/b)/(1/2+3+4): grid with two rows (1 and [2+3+4]) and two columns (a and b).

§ NS at the 5% level.

|| p ≤ 0.001.

of thymulin in thymic epithelium as a function of the histological involution of the thymus and of the nutritional state of children who died in various stages of PEM. We take account of two important factors: whether any infection was present and the zinc content of the thymus. Experimental Zn deficiency or infection can induce thymic involution and a deficiency in cellular immunity (22, 23); in addition, Zn is necessary for the biological activity of thymulin; Thymulin is a Zn metallopeptide (24).

TABLE 2
Histology of the thymus

Nutritional-state group	Time before autopsy*	Weight of thymus*	Weight of thymus as percent of normal*	Hassall corpuscles					Stage of involution				
				Lymphocyte density*			Absent or rare (a)	Moderate number (b)	Large number (c)				
				Fibrosis*	Low (a)	Intermediate (b)	High (c)						
1. Undernourished (n = 18)	4 h 03 min ± 35 min	8.0 ± 1.4	68.5 ± 10.3	21.9 ± 2.7	1	8	9	1	7	10	15	3	0
2. Marasmus (n = 15)	3 h 34 min ± 34 min	1.7 ± 0.2	17.1 ± 2.4	49.7 ± 4.1	8	7	0	7	3	5	2	8	5
3. Kwashiorkor (n = 11)	2 h 51 min ± 42 min	3.0 ± 0.7	19.7 ± 4.7	60.7 ± 3.1	7	4	0	5	4	2	0	5	6
4. Marasmic kwashiorkor (n = 14)	3 h 06 min ± 31 min	1.4 ± 0.2	10.3 ± 1.6	60.6 ± 4.6	13	1	0	6	6	2	0	3	11
Comparisons	F†	F	F	F	f§	f	f	Ra/b+c)/(1/(2+3+4)	Ra/b+c)/(1/(2+3+4)	Ra/b+c)/(1/(2+3+4)			
	1/2/3/4¶	1/2/3/4¶	1/2/3/4¶	1/2/3/4¶	(a/b+c)/(1/(2+3+4)	(a/b+c)/(1/(2+3+4)	(a/b+c)/(1/(2+3+4)						
	1/2+3+4¶	1/2+3+4¶	1/2+3+4¶	1/2+3+4¶									
	2/3/4¶	2/3/4¶	2/3/4¶	2/3/4¶									

* $\bar{x} \pm \text{SEM}$.

† Lymphocyte density in parenchyma: (a) low, some lymphocytes either isolated or grouped in small clusters in connective tissue; (b) intermediate, fairly numerous lymphocytes in a loose parenchyma lacking differentiation between cortex and medulla; and (c) high, a great many lymphocytes in a well-organized tissue.

‡ F: analysis of variance. F 1/2/3/4: comparison of the four groups; F 1/2+3+4: group 1 compared with groups 2, 3, and 4 combined; F 2/3/4: comparison of the three groups 2, 3, and 4.

§ Frequencies, f: Fisher's exact probabilities test. f(a/b)/(1/2+3+4): grid with two rows (1 and [2+3+4]) and two columns (a and b).

¶ NS at the 5% level.

|| p ≤ 0.001.

** p ≤ 0.01.

Subjects, materials, and methods

Clinical and nutritional state

We studied 58 children of both sexes aged 1–4 years (12.1 ± 1.4 mo, $\bar{x} \pm \text{SEM}$) who had died in various stages of nutritional deficiency. According to the Wellcome classification (25), 18 of the children were undernourished, 15 had marasmus, 11 had kwashiorkor, and 14 had marasmic kwashiorkor (Table 1). This classification distinguishes the milder forms of malnutrition (group 1) from the severe forms (groups 2, 3, and 4). A

THYMIC THYMLIN AND ZINC IN MALNUTRITION
TABLE 3
Thymus thymulin content*

Nutritional-state groups	n	Thymulin-positive Hassall corpuscles			Thymulin-positive epithelial cells			Thymulin concentration Index SD
		Number†		Intensity‡	Number†		Intensity‡	
		(a)	(b)	(c)	(a)	(b)	(c)	
1. Undernourished	17	1	5	11	0	15	1	51.6 ± 4.2
2. Marasmus	15	7	5	3	5	3	0	15.8 ± 2.8
3. Kwashiorkor	11	4	6	1	3	4	0	21.7 ± 6.5
4. Marasmic kwashiorkor	14	7	6	1	4	3	0	12.5 ± 3.8
Comparisons		f§	f	f	f	f	f	1/2/3/4¶
		(a/b+c)/(1/(2+3+4)	(a/b+c)/(1/(2+3+4)	(a/b+c)/(1/(2+3+4)	(a/b+c)/(1/(2+3+4)	(a/b+c)/(1/(2+3+4)	(a/b+c)/(1/(2+3+4)	1/2+3+4¶
								2/3/4 †

* The thymus of one undernourished child could not be used in this part of the investigation.

† (a) = absence, score = 0; (b) = moderate, score = 1; (c) = high, score = 2.

‡ (a) = low, score = 1; (b) = moderate, score = 2; (c) = high, score = 3.

§ Frequencies, f: Fisher's exact probabilities test. f(a/b)/(1/2+3+4): grid with two rows (1 and [2+3+4]) and two columns (a and b).

¶ F: analysis of variance. F 1/2/3/4: comparison of the four groups; F 1/2+3+4: group 1 compared with groups 2, 3, and 4 combined; F 2/3/4: comparison of the three groups 2, 3, and 4.

|| p < 0.01.

¶ p < 0.001.

†† NS at the 5% level.

useful control group to exclude more completely the role of infection in thymic involution would have been well nourished, infected children, but it was impossible to find a sufficient number of such children.

Confirmation of the diagnosis of infection

With the permission of the medical and legal authorities of the Hôpital Le Dantec in Dakar, Senegal, the children were autopsied within a few hours of death. The thymus was removed and a systematic pathological examination of the main viscera was performed to provide full data for establishing a diagnosis of infection. It was not always possible to collect bacteriological data.

Examination of thymus glands

Tissue collection and storage. Thymus glands were carefully cut out, dissected, and weighed. To make the investigation age independent, the weights of the glands were also expressed as a percentage of normal for the height of the child according to Stowen's tables (26), which are the most commonly used (8–11). Part of each sample was frozen in liquid nitrogen and then stored at -70 °C for later measurement of Zn and thymulin contents, and another part was fixed in formaldehyde solution and embedded in paraffin wax for histological examination. The time between death and freezing of the sample was on average 3 h 29 min ± 35 min ($\bar{x} \pm \text{SEM}$). All the subsequent analytical determinations were made in a blind fashion.

Histology. The density of lymphoid cells and of Hassall corpuscles in the thymic parenchyma was measured by a conventional microscopical examination ($\times 160$) of 4 µm sections embedded in wax and stained with hematoxylin-eosin. In addition, a microscopic examination with a Quantimet 720 (Imago, Cambridge, UK) image analyzer was performed to quantify the fibrous tissue invading the gland. All this information was used to describe four stages of histological involution (Table 2): normal architecture, moderate involution (well-lubricated thymus, clear distinction between the medulla and

cortex, and lymphocyte density intermediate to high), severe involution (connective tissue composing 50–75% of the gland, loss of corticomedullary differentiation, and lymphocyte density intermediate to low), and extreme involution (connective tissue composing > 75% of the gland and few lymphocytic clusters in atrophied lobules).

Thymulin content. The amount of thymulin in the thymus was evaluated histologically and immunologically (15). In essence, thymulin was recognized in the cytoplasm of productive epithelial cells by means of a rabbit antiserum against synthetic thymulin that had been treated with acetone powders of human organs (liver, stomach, and psoriasis scales) to eliminate any nonspecific binding, particularly to keratin. Staining was performed with a second antiserum (Institut Pasteur, Paris, France) that had been raised in goats against rabbit immunoglobulin G and labeled with fluorescein; it too had been treated with acetone powders of human organs (liver, stomach, and thymus) to eliminate any nonspecific binding, particularly to thymus.

Examination by fluorescence episcopic microscopy ($\times 250$) of 4-µm-thick frozen cut sections treated as described yielded a semiquantitative evaluation of the number of isolated epithelial cells and thymulin-positive Hassall corpuscles in the thymic parenchyma plus the mean intensity of labeling. The numbers of thymulin-labeled structures were graded into three classes: 0 (absence of labeling), 1 (present but scarce), and 2 (same number as in normal young human thymus). If labeled structures could be observed, their fluorescence intensities were scored into three classes: 1 (very difficult to detect), 2 (dim), and 3 (as bright as in normal thymic tissue).

These variables were combined to yield a semiquantitative index of thymulin concentration in the thymic parenchyma. This semiquantitative index was derived by scoring each of the four factors tabulated (Table 3), adding together the products of the number and labeling intensity of FTS-positive Hassall corpuscles and thymulin-positive epithelial cells, and multiplying the result by 100/12. Thus, for a normal subject scored in

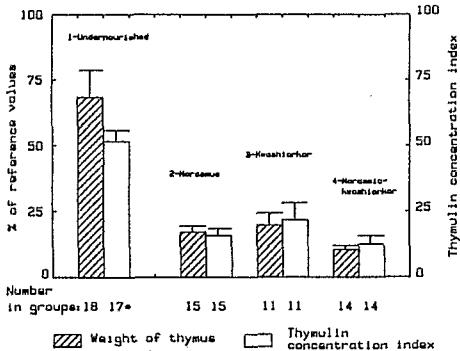


FIG 1. Weight of thymus as percent of normal in the four nutritional groups in 58 children compared with thymulin concentration index. *The thymus of one undernourished child could not be used for the calculation of the thymulin concentration index. †The weight of the thymus is expressed as a percentage of the reference weight of this gland for the height of each child as established by Stoen (26). ‡Thymulin concentration index is calculated as explained in text.

class 2 for the number of elements labeled and in class 3 for the intensity of antithymulin labeling, this gives an index of $([2 \times 3] + [2 \times 3]) \times 100/12 = 100$. This index ranges from 0 in the absence of antithymulin labeling up to 100 in normal children with noninvolved thymus glands rich in epithelial cells and in Hassall corpuscles strongly labeled with antithymulin.

Zinc content. Zn concentrations in the thymus glands were measured by differential impulse polarography with a Tacussel PRG 5 apparatus (Bordeaux, France) on an aliquot of the sample frozen in liquid N and stored at -70°C . Depending on the size of the thymus, 40–680 mg of material was mineralized by heating to dryness with a mixture of 5 mL perchloric acid and 3 mL sulfuric acid and assayed (27). Standardization was performed by the method of measured additions and contamination was avoided as much as possible and taken into account by subtracting reagent blanks.

Statistical methods

Quantitative data (continuous variables) are represented by the mean followed by the SEM. Qualitative data (discontinuous variables) are represented by the distribution frequencies of the observed characteristics. Means were compared by analysis of variance in the case of continuous variables. Comparisons of frequencies and tests of independence were performed by Fisher's exact probabilities test (28) in a 2×2 grid in the case of variables that were discontinuous or were made so by classification. The linear correlation coefficient was used to test for independence between continuous variables.

Results

Clinical and nutritional characteristics

Table 1 shows the homogeneous distribution of ages, sexes, and infections in the four groups of malnourished

children. Note their youth and the frequency of infection (81%) at the time of death. The various infectious diseases observed (of which one individual might have several) were as follows: bronchopneumonia, 39 cases; acute diarrhea, 26 cases; kidney infection, 4 cases; malaria, 7 cases; and other, 10 cases (1 amebic abscess of the liver, 1 anal abscess, 1 febrile jaundice, 2 meningoencephalitis, and 5 recent measles).

The nutritional state of the children examined generally was altered severely. Emaciation was particularly pronounced with an average weight for height of $69.6 \pm 1.4\%$ of normal and a weight for age of $65.0 \pm 2.3\%$. The four nutritional groups appeared to differ very significantly by this criterion ($p < 0.001$) (Table 1). Kwashiorkor differed from the two other severe forms in its relatively less severe weight deficiency, which is partly accounted for by the presence of edema. Deficiencies in stature were only moderate with a height for age of $95.9 \pm 0.9\%$, which did not differ significantly among the four groups studied.

Histology and thymulin content of the thymus as a function of stage of nutritional deficiency

Involution of the thymus, as indicated by its weight deficit and histological data, was clearly greater in the severe forms of PEM (groups 2, 3, and 4) than in the moderate forms (group 1) (Table 2). There did not appear to be any significant difference between the three severe forms (marasmus, kwashiorkor, and marasmic kwashiorkor). The involution was characterized by substantial decrease in the weight of the gland; intralobular and interlobular invasion of connective tissue; consistently reduced lymphocyte population in the parenchyma, sometimes extending to its total disappearance from the lobules; nearly universal loss of distinction between cortex and medulla; fewer or no Hassall corpuscles; and dilation and varying degrees of necrosis of remaining Hassall corpuscles.

TABLE 4
Zinc concentration in the thymus glands of well-fed humans and animals

Origin	Zinc concentration		
	$\mu\text{g Zn/g}$	thymus	$(\mu\text{mol/g})$
Human (young adult)	20.2 (0.309)	1	Differential impulse polarography (27)
Human (young adult)	14.0 (0.214)	1	Differential impulse polarography (27)
Calf	17.9 (0.274)	1	Differential impulse polarography (27)
Pig (young adult)	16.8 (0.257)	1	Differential impulse polarography (27)
Mouse	17.9 (0.274)	2	Inductively coupled plasma atomic spectrometry (28)

TABLE 5
Zinc concentration in the thymus in relation to nutritional state*

Nutritional-state groups	n	Weight of thymus	Infection		Zinc
			Absent (a)	Present (b)	
		g			$\mu\text{g/g} (\mu\text{mol/g})$
1. Undernourished	17	7.8 ± 1.6	4	13	$24.5 \pm 3.2 (0.375 \pm 0.049)$
2. Marasmus	13	1.7 ± 0.3	2	11	$22.6 \pm 3.7 (0.346 \pm 0.057)$
3. Kwashiorkor	10	3.1 ± 0.7	1	9	$21.2 \pm 3.2 (0.324 \pm 0.049)$
4. Marasmic kwashiorkor	14	1.4 ± 0.2	2	12	$22.3 \pm 3.0 (0.341 \pm 0.046)$
Comparisons			F†	F‡	F
			1/2/3/4§	(a/b)/1/2+3+4	1/2/3/4
			1/2+3+4§	1/2+3+4	1/2+3+4
			2/3/4	2/3/4	2/3/4

* The thymus glands of four children (one undernourished, two with marasmus, and one with kwashiorkor) could not be used for measurements of zinc.

† F: analysis of variance. F 1/2/3/4: comparison of the four groups; F 1/2+3+4: group 1 compared with groups 2, 3, and 4 combined; F 2/3/4: comparison of the three groups 2, 3, and 4.

‡ Frequencies; f: Fisher's exact probabilities test; f(a/b)/(1/2+3+4): grid with two rows (1 and [2+3+4]) and two columns (a and b).

§ p ≤ 0.01.

|| NS at the 5% level.

These observations are well summed up by the stage of histological involution. Almost all the children suffering from severe forms of malnutrition (groups 2, 3, and 4) had seriously involved thymus glands (severe-to-extreme involution) vs 17% among the children of group 1.

Table 3 shows the major differences in anti-FTS labeling of the epithelial elements of the thymic parenchyma between group 1 and groups 2, 3, and 4. The thymus glands of group 1 almost always contained isolated epithelial cells and Hassall corpuscles that clearly took up

TABLE 6
Zinc concentration in the thymus in relation to body weight for height*

Weight for height (wt/ht)	n	Zinc		
			% of normal	$\mu\text{g/g} (\mu\text{mol/g})$
(a) wt/ht ≥ 80	11			
(b) 70 ≤ wt/ht < 80	14			
(c) 60 ≤ wt/ht < 70	19			
(d) wt/ht < 60	10			
Comparisons			F‡	
			a/b/c/d§	
			a/b+c+d§	
			b/c/d§	

* The thymus glands of four children (one undernourished, two with marasmus, and one with kwashiorkor) could not be used.

† $\bar{x} \pm \text{SEM}$.

‡ F: analysis of variance. F 1/2/3/4: comparison of the four groups; F 1/2+3+4: group 1 compared with groups 2, 3, and 4 combined; F 2/3/4: comparison of the three groups 2, 3, and 4.

§ NS at the 5% level.

the antithymulin label whereas in groups 2, 3, and 4 these features were less common and less intensely labeled or absent. The concentration of thymulin in the thymic parenchyma, evaluated by the histological criteria previously described, sums up these observations. Expressed as a percentage of the score attainable in normal children, the thymulin concentration appeared to be relatively conserved in the mildly malnourished children with a mean score > 50% whereas it was very much lower in the three groups of severely malnourished children. Figure 1 shows, in addition, the close parallel between this concentration and the involution of the thymus by weight. Finally it should be noted that in 27% of the severe forms of PEM, in which the thymus was extremely atrophied, no antithymulin labeling was seen, which suggests that these thymus glands lacked this hormone.

Zinc content of the thymus as a function of degree of nutritional deficiency

Values obtained from two well-fed young-adult thymus glands are in good accord with published references values (29, 30) and confirm the validity of our method of Zn determination (Table 4). Tables 5 and 6 show that there was no change in the concentration of Zn in the thymus as a function of either the clinical form of PEM or the weight deficit. The mean concentration of Zn for all 54 thymus glands examined in this part of the investigation was $22.8 \pm 1.7 \mu\text{g/g}$, a value similar to those found in human or animal (Table 4).

Zinc content of the thymus as a function of degree of involution and of thymulin content

Tables 7 and 8 show that there were no significant differences among thymus Zn concentrations as a func-

TABLE 7
Zinc concentration in the thymus in relation to weight of the gland*

Weight of thymus	n	Zinc
g		μg/g (μmol/g)†
(a) thymus < 2	27	21.6 ± 2.4 (0.330 ± 0.037)
(b) 2 ≤ thymus ≤ 5	15	22.3 ± 2.6 (0.341 ± 0.040)
(c) thymus > 5	12	26.1 ± 4.0 (0.399 ± 0.061)
Comparisons		F‡ a/b/c§

* The thymus glands of four children (one undernourished, two with marasmus, and one with kwashiorkor) could not be used.

† $\bar{x} \pm$ SEM.

‡ F: analysis of variance. F 1/2/3/4: comparison of the four groups; F 1/2+3+4: group 1 compared with groups 2, 3, and 4 combined; F 2/3/4: comparison of the three groups 2, 3, and 4.

§ NS at the 5% level.

tion of the weight deficiency of the thymus or of the concentration of thymulin in the thymic parenchyma.

Effects of infection

None of the variables studied in this investigation was affected by the presence of infection except for the thymus Zn concentration. Fifty-four children were studied in this regard. The thymus glands of the 10 uninfected children differed from the rest in their significantly higher Zn concentration ($31.5 \pm 4.7 \mu\text{g/g}$ vs $20.8 \pm 1.6 \mu\text{g/g}$, $p < 0.01$).

Effects of the interval before autopsy

None of the variables studied, in particular the anti-thymulin labeling, appeared to be significantly related to the time between the child's death and the freezing and fixing of the thymus. The longest delays are not statistically correlated with the lowest antithymulin labeling, which suggests that the intrathymic thymulin was not degraded within these relatively short periods (Table 2).

Discussion

Our results confirm the involution of the thymus in children who die in a state of PEM. The very severe thymic damage in severe forms of PEM is accompanied by an at least equally severe decrease in the thymulin content of the gland. These observations are in accord with the idea of a substantial decrease in the contribution of thymulin to the lymphocyte-differentiating action of the thymus. They also suggest that there is some damage to the thymic epithelium, of which thymulin is a functional marker.

Our findings agree completely with those of Chandra (20), who showed a substantial decrease in circulating thymulin in severely malnourished children. They also agree with the observations of Olusi (18) and Jackson (19), who showed the sensitivity of peripheral lymphocytes of severely malnourished children to thymosin and

thymopoietin; this suggests a deficiency of these two factors, which are also produced by the thymic epithelium (13). On the other hand, our results conflict with those of Maire et al (21), who did not find any changes in thymulin activity as a function of the nutritional state in a population of hospitalized children in the same hospital in Dakar.

The main difference between the work of those authors and our work is that we made our observations exclusively on dead children. Any comparison with living children, even seriously ill ones, must therefore be interpreted cautiously.

In addition, the immunohistological technique used in this investigation specifically identified the thymulin molecule in thymic parenchyma. Biological estimation of circulating thymulin by the technique of azathioprine-sensitive rosettes may be affected by an allogeneic factor secreted by activated T lymphocytes (31) among other things. Maire et al (21) did not exclude this hypothesis because most of the children in their investigation had infections, in contrast with those studied by Chandra (20). Most of the children we studied had infections too and yet in cases of severe malnutrition had very low thymulin concentrations of thymulin.

It is plausible that the degenerative process might have a similar effect on other lymphocyte-differentiating hormones secreted by thymic epithelium, in particular thymosin I and thymopoietin. In fact, Savino (32) showed that these are produced in humans by the same epithelial cells as those producing thymulin.

In our investigation, a Zn deficiency in the thymus does not on its own explain the observed facts. There was no significant relationship between the Zn concentration and either the nutritional state or the degree of involution or thymulin content of the thymus. Obviously this does not rule out the possibility that Zn deficiency may have some effect outside the thymus because this metal in particular is necessary for the lymphocyte-differenti-

TABLE 8
Zinc concentration in the thymus in relation to thymic thymulin content*

Thymus FTS concentration	n	Zinc
%		μg/g (μmol/g)†
(a) Thymulin < 10	16	23.7 ± 3.4 (0.363 ± 0.052)
(b) 10 ≤ thymulin ≤ 40	21	21.6 ± 3.0 (0.330 ± 0.046)
(c) Thymulin > 40	17	23.4 ± 2.0 (0.358 ± 0.031)
Comparisons		F‡ a/b/c§

* The thymus glands of four children (one undernourished, two with marasmus, and one with kwashiorkor) could not be used.

† $\bar{x} \pm$ SEM.

‡ F: analysis of variance. F 1/2/3/4: comparison of the four groups; F 1/2+3+4: group 1 compared with groups 2, 3, and 4 combined; F 2/3/4: comparison of the three groups 2, 3, and 4.

§ NS at the 5% level.

THYMIC THYMLIN AND ZINC IN MALNUTRITION

ating activity of thymulin, the active Zn-complexed form of the hormone. The presence of a Zn deficiency perhaps associated with PEM depends on the nutritional environment, which differs from one country to another. In the Dakar region Zn deficiency does not seem to be very great, as Maire et al (21) showed by measurements of Zn concentrations in blood.

Our results are not, however, incompatible with those of Golden and Jackson (33), who showed the beneficial effects of Zn supplements on size of the thymus evaluated by radiography of malnourished children. Zn does in fact favor protein synthesis and cell multiplication (34). In fact, there may be several independent causes of thymic atrophy, including low Zn, PEM, infection, and steroids, of which only PEM was shown conclusively for this Senegalese cohort.

In this investigation infection apparently had no direct effect on thymic involution. The only relationship between infection and the other variables examined is with the Zn concentration in the thymus. The thymus glands of noninfected subjects were in fact significantly richer in Zn. Zn, like iron, can rapidly disappear from plasma in many infections to be accumulated primarily in the liver. This redistribution could have a possible value as a host defensive mechanism (35, 36). Other alterations in Zn metabolism can be associated with infection, such as diminished dietary intake or increased losses of body Zn. There are no major body storage depots for Zn so that deficiency seems to be usual (22, 23).

This investigation provides direct evidence that the thymic involution seen in the course of PEM is accompanied by altered content of thymulin. Insofar as the thymulin content can be considered representative of the lymphocyte-differentiating function of the thymic epithelium, this functional change is probably one of the main causes of the deficiency in cell-mediated immunity.

It would therefore be of interest to use therapeutic means to alleviate the deficiency of thymic functions in severely malnourished children. In some severe forms of PEM, at least on a short-term basis, replacement therapy using synthetic thymic hormones, might be used along with the very important nutritional measures. This has been performed successfully in certain severe congenital or acquired immune deficiencies (37, 38).

We thank R Dardelin and P Tankosic for their valuable help in the pathology studies of the thymus.

References

- Chandra RK. Immunology of nutritional disorders. London: Edward Arnold 1980.
- Suskind RM. Malnutrition and the immune response. New York: Raven Press, 1977.
- Chandra RK, Gupta S, Singh H. Inducer and suppressor T cell subsets in protein-energy malnutrition: analysis by monoclonal antibodies. Nutr Res 1982;2:21-6.
- Chandra RK. T and B lymphocyte subpopulation and terminal deoxynucleotidyl transferase in undernutrition. Acta Paediatr Scand 1979;68:841-5.
- Platt BS, Stewart RJC. Experimental protein-calorie deficiency: histopathological changes in the endocrine glands of pigs. J Endocrinol 1967;38:121-43.
- Watts TH. Thymus weights in malnourished infants. J Trop Pediatr 1969;15:155-8.
- Mugerwa JW. The lympho-reticular system in kwashiorkor. J Pathol 1971;105:105-8.
- Smythe P, Schonland M, Brereton-Stiles GG, et al. Thymolymphatic deficiency and depression of cell-mediated immunity in protein-calorie malnutrition. Lancet 1971;2:939-43.
- Purtillo DT, Connor DH. Fatal infections in protein-calorie malnourished children with thymo-lymphatic atrophy. Arch Dis Child 1975;50:149-52.
- Maamouri MT, Khadraoui S, Hamza B, et al. Les organes thymo-lymphatiques dans les états de malnutrition protéino-calorique. Etude anatomique de 15 observations. Arch Anat Cytol Pathol 1976;24:37-42.
- Aref GH, Adel-Aziz A, Elaraby II, et al. A post-mortem study of the thymolymphatic system in protein-energy malnutrition. J Trop Med Hyg 1982;85:109-14.
- Mittal A, Woodwar B. Thymic epithelial cells of severely undernourished mice. Accumulation of cholesterol esters and absence of cytoplasmic vacuoles. Proc Soc Exp Biol Med 1985;178:385-91.
- Dardenne M, Bach JF. Thymic hormones. In: Kendall MD, ed. The thymus gland. London: Academic Press, 1981:113-31.
- Savino W, Dardenne M, Papernik M, et al. Thymic hormone containing cells. Characterization and localization of serum thymic factor in young mouse thymus studied by monoclonal antibodies. J Exp Med 1982;156:628-33.
- Jambon P, Montagne P, Benet MC, et al. Immunohistologic localization of factor thymique sérique (FTS) in human thymic epithelium. J Immunol 1981;127:2055-9.
- Bach JF, Dardenne M, Pleau JM, et al. Isolation, biochemical characteristics and biological activity of a circulating thymic hormone in the mouse and in the human. Ann NY Acad Sci 1975;249:186-210.
- Bach JF, Bach MA, Blanot D, et al. Thymic serum factor. Bull Inst Pasteur 1978;76:325-98.
- Olusi SO, Thurman GB, Goldstein AL. Effect of thymosin on T-lymphocyte rosette formation in children with kwashiorkor. Clin Immunol Immunopathol 1980;15:687-91.
- Jackson TM, Zaman SN. The in vitro effect of the thymic factor thymopoietin on a subpopulation of lymphocytes from severely malnourished children. Clin Exp Immunol 1980;39:717-21.
- Chandra RK. Serum thymic hormone activity in protein-energy malnutrition. Clin Exp Immunol 1979;38:228-30.
- Maire B, Wade S, Bleiberg F, et al. Absence of variation in factor thymique sérique activity in moderately and severely malnourished Senegalese children. Am J Clin Nutr 1982;36:1129-33.
- Beisel WR. Single nutrients and immunity. Am J Clin Nutr 1982;35:417-68.
- Chandra RK, Newberne PM. Nutrition, immunity and infection: mechanisms of interactions. New York: Plenum Publishing Corporation, 1977.
- Dardenne M, Pleau JM, Nabarra B, et al. Contribution of zinc and other metals to biological activity of the serum thymic factor (FTS). Proc Natl Acad Sci USA 1982;79:5370-3.
- Wellcome Trust Working Party. Classification of infantile malnutrition. Lancet 1970;2:302-3.
- Stowens D. Growth and development in pediatric pathology. Baltimore, Williams & Wilkins, 1966.
- Huin MF, Burnel D. Dosage du zinc dans l'eau et certains milieux

- biologiques par polarographie impulsionnelle. Ann Falsifications Expertise Chim 1984;83:587-93.
28. Fischer RA. Statistical methods for research workers. Edinburg: Oliver and Boyd, 1950.
 29. Lyengar GV, Kollmer WE, Bowen HJM, comps. The elemental composition of human tissues and body fluids. A compilation of values for adults. Weinheim: Verlag Chemie, 1978.
 30. Suzuki KT, Yamada YK, Shimizu F. Essential metals and metallothionein in cadmium induced thymic atrophy and splenomegaly. Biochem Pharmacol 1981;30:1217-22.
 31. Dardenne M, Bach JF. Demonstration and characterization of a serum factor produced by activated T cells. Immunology 1977;33: 643-51.
 32. Savino N, Dardenne M. Thymic hormone containing cells. IV Immunohistologic evidence for the simultaneous presence of thymulin, thymopoietin and thymosin τ in normal and pathological human thymus. Eur J Immunol 1984;14:987-91.
 33. Golden MHN, Jackson AA, Golden BE. Effect of zinc on thymus of recently malnourished children. Lancet 1977;2:1057-9.
 34. Golden MHN, Golden BE. Trace elements potential importance in human nutrition with particular reference to zinc and vanadium. Br Med Bull 1981;37:31-6.
 35. Beisel WR. Magnitude of the host nutritional responses to infection. Am J Clin Nutr 1977;30:1236-47.
 36. Powanda MC. Changes in body balances of nitrogen and other key nutrients: description and underlying mechanisms. Am J Clin Nutr 1977;30:1254-68.
 37. Bordigoni P, Faure G, Bens MC, et al. Improvement of cellular immunity and IgA production in immunodeficient children after treatment with synthetic serum thymic factor (FTS). Lancet 1982;2:293-7.
 38. Faure G, Bordigoni P, Bens MC, et al. Thymic factor therapy of primary T-lymphocyte immunodeficiencies. In: Byron NA, Hobbs JR, eds. Thymic factor therapy. New York: Raven Press, 1984: 147-53. (Serono symposia publication #16.)