Thymulin (factor thymocyte serique) and zinc contents of the thymus glands of malnourished children 1-3

Bernard Jambon, PhD; Olivier Ziegler, MD; Bernard Maire, PhD; Marie-France Nutin, PhD; Gérard Parent, MD; Mohamadou Fall, MD; Daniel Brantel, PhD; and Jean Duselille, 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 (factor thymic serique, or FTS) and of Zn in the thymus glands of 55 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.


KEY WORDS
Protein-energy malnutrition, factor thymocyte serique, 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 clearly established (16, 17). A change in the capacity of the thymic epithelium to produce the hormononal factors for lymphocyte differ-
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 zinc deficiency or infection can induce thymic involution and a deficiency in cellular immunity (22, 23); in addition, zinc is necessary for the biological activity of thymulin; Thymulin is a Zn metalloprotein (24).

Subjects, materials, and methods

Clinical and nutritional state

We studied 58 children of both sexes aged 1–4 y (1.2 ± 1.4 mo, F = 24, M = 34) who had died in various stages of nutritional deficiency. According to the Wellcome classification (25), 18 of the children were undernourished, 15 had marmasik, 11 had kwashiorkor, and 14 had marrasil kwashiorkor (Table 2). This classification distinguishes the milder forms of malnutrition (group 1) from the severe forms (groups 2, 3, and 4). A 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.

Conformation of the diagnosis of infection

With the permission of the medical and legal authorities of the Hospital Le Ducrocq in Dakar, Senegal, the children were autopsied within a few hours of death. The thymus was removed and a systematic pathologic examination of the main vessels 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 Snowdon's tables (26), which are the most commonly used (8–11). Part of each sample was frozen in liquid nitrogen and then lyophilized. The remainder was fixed for later measurement of Zn and thymulin concentrations, and for histochemistry. The time between death and freezing of the sample was on average 3.2 h, with a minimum of 2 min (± 3.5 in F = 2 SEM). All the subsequent histological investigations were made in a blind fashion.

Histology. The density of lymphoid cells and of Hassall corpora in the thymic parenchyma was measured by a conventional microscopic examination (X100) of 4–6 sections embedded in paraffin and stained with hematoxylin-eosin. In addition, a semiquantitative index was derived by scoring each of the four factors (Table 2) normal architecture, moderate involution (well-preserved Zn corpuscles and thymulin-positive Hassall corpuscles in the thymic parenchyma plus the mean intensity of labeling). The index was calculated as follows:

\[
\text{Index (I)} = \frac{100}{4} \times \left( \frac{1}{2} \times \text{Low} + \frac{1}{2} \times \text{Medium} + \frac{3}{4} \times \text{High} + \frac{1}{4} \times \text{Extreme} \right)
\]

where Low = absence of labeling, Medium = present but scarce, High = present but dense, and Extreme = present and dense. The paraffin sections were stained with hematoxylin-eosin, thionine, and Giemsa. 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 acetoacetic powders of human organs (liver, stomach, and thymus) 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 acetoacetic powders of human organs (liver, stomach, and thymus) to eliminate any nonspecific binding, particularly to keratin.

Examination by fluorescence microscopic microscopy (X250) of 4–6 thick frozen sections cut serially treated as described yielded a semiquantitative evaluation of the number of isolated epithelial cells and thymulin-positive Hassall corpora in the thymic parenchyma plus the mean intensity of labeling. The numbers of thymulin-labeled strata 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.
concentration index

The thymus of

intensity of antithymulin labeling, this gives an index of

Statistical

in the absence of antithymulin labeling up to

class 2 for the number of elements labeled and in class

by subtracting reagent blanks.

ple frozen in liquid N and stored at

size of the thymus,

formation was avoided

content.

FIG 1. Weight of thymus as percent of normal in the four nutritional

groups in 58 children compared with thymulin

as a function of stage of nutritional deficiency

Involvement of the thymus, as indicated by its weight
deficit and histological data, was clearly greater in the se-

vere forms of PEM (groups 2, 3, and 4) than in the mod-

erate forms (group 1) (Table 2). There did not appear to

be any significant difference between the three severe

forms (marasmus, kwashiorkor, and marasmic kwashi-

orkor). The involution was characterized by substantial
decrease in the weight of the gland; intralobular and in-
terlobular invasion of connective tissue; consistently re-
duced lymphocyte population in the parenchyma, some-
times extending to its total disappearance from the lob-

ules; nearly universal loss of distinction between cortex

and medulla; fewer or no Hassall's corpuscles; and dilation
and varying degrees of necrosis of remaining Hassall cor-

puscles. 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

serious thymus glandular involvement (severe-to-

extreme involution) vs 17% among the children of group 1.

Table 3 shows the major differences in anti-FTS label-
ing of the epithelial elements of the thymic parenchyma

between group I and groups 2, 3, and 4. The thymus glands of
group 1 almost always contained isolated ep-

ithelial cells and Hassall corpuscles that clearly took up

Zinc concentrations in the thymus glands of well-fed humans and
animals

TABLE 4

Zinc concentration in the thymus glands of well-fed humans and anim-
als

<table>
<thead>
<tr>
<th>Zinc concentration</th>
<th>n</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human (young adult)</td>
<td>16.8 (0.257)</td>
<td>Differential impulse polarography (27)</td>
</tr>
<tr>
<td>Human (young adult)</td>
<td>17.9 (0.274)</td>
<td>Differential impulse polarography (27)</td>
</tr>
<tr>
<td>Pig (young adult)</td>
<td>19.7 (0.302)</td>
<td>Differential impulse polarography (27)</td>
</tr>
<tr>
<td>Human (young adult)</td>
<td>14.0 (0.214)</td>
<td>Differential impulse polarography (27)</td>
</tr>
<tr>
<td>Human (young adult)</td>
<td>20.2 (0.309)</td>
<td>Differential impulse polarography (27)</td>
</tr>
</tbody>
</table>

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 infections (81%) at the time of death. The various infectious dis-

cases observed (of which one individual might have sever-

eral) 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 feline jaundice, 2 meningococcemia,

and 5 recent measles).

The nutritional state of the children examined gener-

ally was altered severely. Emaciation was particularly

pronounced with an average weight for height of 69.6

± 1.4% of normal and a weight for age of 65.0 ± 2.9%.

The four nutritional groups appeared to differ very signif-

icantly by this criterion (p < 0.001) (Table 1). Kwashi-

orkor differed from the other two severe forms in its rela-
tively less severe weight deficiency, which is partly ac-
counted for by the presence of edema. Deficiencies in

stature were only moderate with a height for age of 95.9

± 0.9%, which did not differ significantly among the four

groups studied.

Histology and thymus content of the thymus as a

function of sex

These observations are well summed up by the stage of

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have seriously involuted thymus glands (severe-to-

involution) and showed no change in the concentration of

Zn for the weight deficit. The mean concentration of Zn for

the thymi is as indication of degree

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.

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 24 thymus glands examined in this part of the investi-
gation was 22.8 ± 1.7 µg/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 thymus content

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

of age.
TABLE 7

Zinc concentration in the thymus in relation to weight of the gland

<table>
<thead>
<tr>
<th>Weight of thymus</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>g</td>
<td>µg/g</td>
</tr>
<tr>
<td>(a) thymus ≤ 2</td>
<td>27</td>
</tr>
<tr>
<td>3 thymus ≤ 5</td>
<td>23.3</td>
</tr>
<tr>
<td>(b) thymus &gt; 5</td>
<td>12</td>
</tr>
</tbody>
</table>

Comparisons

<table>
<thead>
<tr>
<th></th>
<th>F (p)</th>
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<tbody>
<tr>
<td>thymus ≤ 2</td>
<td>2.20</td>
</tr>
<tr>
<td>3 thymus ≤ 5</td>
<td>2.34</td>
</tr>
</tbody>
</table>

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

F = SEM.

Thymic thymulin and zinc in malnutrition

Table 7 shows the zinc concentration in the thymus in relation to weight of the gland. The zinc concentration was significantly higher in thymuses of severely malnourished children than in thymuses of control children. This suggests a deficiency of these two factors, which are also produced by the thymic epithelium. In the thymus of severely malnourished children, the thymus hormone thymulin was found to be significantly lower than in the thymus of normal children. This difference was most pronounced in children who died in a state of kwashiorkor. The very severe zinc deficiency in these children who die in a state of PEM is not necessarily related to the thymic involution seen in the course of PEM. The only relationship between the thymic function and the degree of involution is that the thymus of noninfected subjects was in fact significantly richer in Zn than in infected subjects. This suggests that the thymus glands of severely malnourished children are undernourished in the same way as the thymus glands of noninfected subjects.

Our findings agree completely with those of Chandra (25), who showed a substantial decrease in circulating thymulin in severely malnourished children. They also agree with the observations of Olus (18) and Jackson (19), who showed the sensitivity of peripheral lymphocytes of severely malnourished children to thymopoeitin; this suggests a deficiency of these two factors, which are also produced by the thymic epithelium. 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 the beneficial effects of Zn supplements on size of the thymus evaluated by radiography of malnourished children. Zn does not influence thymic involution 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 than in infected subjects, such as diminished dietary intake or increased losses of body Zn. These facts, together with body stores being a source for Zn so that deficiency seems to be usual (22, 23).

Our results confirm the involution of the thymus in children who die in a state of PEM. Our findings agree completely with those of Chandra et al. (25), who showed the beneficial effect of thymulin to the lymphocytedifferentiating action of thymulin (31). 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.

In this investigation we have not been able to show the sensitivity of peripheral lymphocytes to the thymic hormone thymulin. This suggests that the thymic function, which is not necessarily related to 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 lymphocytodifferentiating 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 function 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. Daradini and P. Tinkoff for their valuable help in the laboratory work and in the histopathologic examination of the thymus.

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Zinc status of healthy elderly adults: response to supplementation \(^1, 2\)

*Christine A. Swanson, PhD; Robert Munsouriun, BS; Henri Dirren, PhD; and Charles-Henri Rapin, MD*

**ABSTRACT** The zinc status of 33 healthy elderly subjects was evaluated. The dietary Zn intake estimated by 24-h recall was 9.2 mg/d and 65% of subjects had intakes less than two-thirds of the RDA. Mean serum Zn concentration (13.0 \(\mu\)mol/L) and urinary Zn excretion (7.0 \(\mu\)mol/d) were normal. The Zn content of platelets, mononuclear cells, and polymorphonuclear cells was 5.8, 147, and 135 pmol/10^9 cells, respectively. Seventeen subjects were supplemented for 28 d with 30 mg Zn/d. The mean concentration of Zn in serum and urine increased 24% and 2.5-fold, respectively. Zn content of platelets and leukocytes did not change with Zn supplementation. The concentration of visceral proteins (ie, albumin, prealbumin, transferrin, and retinol-binding protein) and immunoglobulins (ie, IgG, IgA, and IgM) did not change with Zn supplementation. The data indicate that aging per se does not necessarily imply poor Zn status.


**KEY WORDS** Zinc, aged, zinc status, zinc supplementation

**Introduction**

Despite the importance of adequate nutrition for the maintenance of health, specific nutrient needs of elderly adults have not been studied extensively. Poor wound healing and compromised immune function in elderly adults, for example, may be associated with poor Zn nutriture \(^1, 2\). The etiology of suboptimal Zn nutriture in elderly persons has been related to changes in eating habits that may reduce both the amount and bioavailability of dietary Zn \(^3\). Age-associated changes in physical and comorbid conditions of medication may also place elderly individuals at greater risk of poor Zn status.

The prevalence of poor Zn nutriture in older adults is unknown. Zn status is difficult to assess \(^4\); no single index has adequate sensitivity and specificity. The concentration of Zn in serum is reported to decrease slightly with age \(^5\) but circulating levels of Zn do not necessarily reflect tissue levels. The concentration of Zn in blood cells may provide an index of whole-body Zn nutriture \(^6\). Leukocyte Zn concentration, for example, decreased in adult men fed Zn-restricted diets \(^7\). Alkaline phosphatase is a Zn metalloenzyme and Zn supplementation increased alkaline phosphatase activity of individuals depleted of Zn during prolonged total parenteral nutrition \(^8\). Zn supplementation produced a similar response in individuals with Zn deficiency associated with acrodermatitis enteropathica \(^9\). Protein synthesis processes require Zn \(^10\) and circulating levels of serum proteins provide indirect indices of Zn status. In a study of patients receiving TPN, Zn deficiency was associated with reduced levels of serum proteins \(^11\); concentrations of transferrin and prealbumin in serum decreased during Zn deficiency and increased with Zn supplementation. Wahlqvist et al \(^12\) reported that Zn supplementation resulted in increased concentration of serum albumin in hyperalimented aged persons.

The purpose of this study was to broadly evaluate Zn status of healthy elderly persons, to accumulate normative data for this population group, and to identify biochemical indices responsive to Zn supplementation.

**Methods**

**Subjects and protocol**

The study participants were residents of a public housing facility in Geneva, Switzerland. The criteria for study enrollment included 1) age > 64 y, 2) absence of debilitating illness, 3) willingness to provide information for a dietary survey, 4) donation of one 30-mL blood sample, and 5) collection of a