Summary

A comparative study of the level of 4 plasma proteins in malnutrition shows that albumin has low sensitivity, transferrin has intermediate and the TBPA-RBP complex has the highest sensitivity to an alteration in the nutritional status. According to protein and/or iron deficiency, the synthesis of transferrin seems to be submitted to contradictory impulses which partially invalidates this test as a reliable index for estimating protein depletion alone. On the contrary, the components of the TBPA-RBP complex respond together and in a parallel direction to protein deficiency. The high degree of sensitivity of TBPA and RBP to an inadequate protein intake is apparently related to their rapid turnover rate and to their unusual richness in tryptophan, which is known to play a key role in the control of protein synthesis. Measurement of TBPA (or RBP) is proposed as a method for the detection of pre-kwashiorkor and early marasmus.

Introduction

Among the battery of tests available for evaluating protein-calorie malnutrition (PCM), the usefulness of transferrin has been documented in various parts of Africa [1–5]. More recently, the measurement of thyroxine-binding prealbumin (TBPA) has been proposed for a similar purpose [6]. Besides its function in thyroxine transport, TBPA serves as a carrier protein for retinol-binding protein (RBP), the specific protein for vitamin A alcohol transport [7,8]. It has been shown that the TBPA-RBP complex drastically drops in the
acute stage of kwashiorkor and returns to normal in keeping the same molar ratio during nutritional rehabilitation [9,10].

The present study was undertaken in order to compare the respective sensitivity of albumin, transferrin, TBPA and RBP in the assessment of nutritional status.

Material and Methods

Thirty-seven Senegalese children, aged 18-30 months, with a peak incidence of two years, were admitted to the metabolic ward for this survey. They presented all the clinical features of severe PCM: weight loss, growth failure, hair discoloration, skin lesions, diarrhea and swollen limbs. According to the Wellcome Working Party classification [11], fifteen among them had an expected weight for age situated between 80% and 60% of the Boston standard weight for age (50th percentile) and were regarded as kwashiorkor of recent onset with limited height deficit [12]. The remaining twenty-two children with a percentage of expected weight for age situated below 60% of the Boston standard [12] were considered as marasmic kwashiorkor. This latter group was also significantly more retarded in height, implying the effect of prolonged malnutrition [13]. None of the children presented clinical symptoms of vitamin A-deficiency nor apparent evidence of infections or parasitism. A certain degree of anaemia, substantiated by the red blood cell count and hemoglobin concentration, was discovered in all of them. In the appearance of anaemia, the possible interference of glucose-6-phosphate dehydrogenase deficiency and of hetero-/homozygous sickle cell hemoglobin (HbAS/HbSS) was investigated and subsequently discarded, suggesting therefore that anaemia was mainly the result of nutritional deficiencies.

Dietary treatment was started immediately after admission and consisted of increasing quantities of semi-skimmed milk (“Nido”, Nestle) and a commercial mixture of amino acids and oligopeptides (“Nesmida”, Nestle). No detectable amount of vitamin A was found in the latter hydrolysate. The vitamin A content of the Nido skim powder was evaluated at 12.1 μg ± 1.9 (range 8.7 to 16.3)/100 g edible powder. Nesmida hydrolysate was given until the tenth day of the survey. After the tenth day, only Nido constituted the regimen which reached a mean plateau intake of 150 g edible powder/day, or about 3.5 g protein and 150 kcal/kg body weight per day. A mineral supplement of K and Mg was added to the diet in keeping with the patients individual requirements [6]. No additional supply of drugs or vitamins was administered to the children. None was given blood transfusion, and all recovered normally.

Venipunctures were made on admission (day 1) and at weekly intervals during the period of appropriate refeeding (day 8, day 15, day 22). Measurement of each of the 4 proteins was performed on the same blood sample. Serum protein level was estimated by the biuret reaction. Serum albumin concentration was evaluated after protein electrophoresis with the phoroslide system (Millipore Corp., Bedford, U.S.A.). Measurement of transferrin, TBPA and RBP was achieved by the method of radial immunodiffusion. Partigen specific immunoplates were provided by Behringwerke AG (Marburg a/Lahn, West Germany).
Plasma retinol concentrations and vitamin A in the regimen were estimated by the micromethod of Bessey et al. [14].

The normal group was composed of 30 healthy Senegalese children most of them belonging to families of medical employees. The children had the same age as the children in the malnourished group. Statistical calculations were achieved according to Snedecor and Cochran [15].

Results

Mean values ± S.D. recorded in the control group (n = 30) are summarized in Table I. Mean values ± S.D. obtained in the malnourished group (n = 37) at the successive steps of nutritional rehabilitation are collected in Table II, which gives also the range and the percentage of each mean by comparison with the control group. Fig. 1 shows the general evolution of the individual values of each protein.

The mean average for serum albumin on admission (1.74 ± 0.39 g/100 ml, or 52.8% of the control) rose progressively to 3.22 ± 0.37 g/100 ml, or 97.8% of the control on day 22.

The mean average for serum transferrin on admission (131 ± 57 mg/100 ml, or 38.7% of the control) reached 445 ± 167 mg/100 ml, or 131.3% of the control value on day 22.

TBPA and RBP mean plasma levels ran parallel during recovery. Both proteins doubled their plasma concentration after a week, and tripled it after two weeks of dietary therapy. From day 1 to day 22, TBPA increased from 6.38 ± 1.89 (28.5% of the control) to 22.31 ± 3.47 mg/100 ml (99.8% of the control), whereas RBP gradually increased from 1.62 ± 0.72 (31.9% of the control) to 5.41 ± 0.87 mg/100 ml (106.7% of the control).

The mean values recorded on day 22 for serum albumin, TBPA and RBP were not significantly different from the corresponding mean values in the control group. On the contrary, the mean value calculated on day 22 for serum transferrin was significantly higher than in the control group. (0.01 > p > 0.001).

In the PCM group, the mean plasma retinol level was 11.43 ± 4.26 μg/100 ml on admission and rose gradually to 42.07 ± 9.71 μg/100 ml on day 22. Plasma retinol levels on day 22 were between 3 and 4 times the starting value and were not significantly different from the control group.

The standard group was characterized by a red blood count averaging 3.63 × 10⁶ ± 0.29 × 10⁶/mm³ (range 3.4 × 10⁶ to 4.1 × 10⁶) and blood hemoglobin

<table>
<thead>
<tr>
<th>Albumin (g/100 ml)</th>
<th>Transferrin (mg/100 ml)</th>
<th>TBPA (mg/100 ml)</th>
<th>RBP (mg/100 ml)</th>
<th>Retinol (μg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.29 ± 0.31</td>
<td>338 ± 45</td>
<td>22.4 ± 3.78</td>
<td>5.1 ± 1.37</td>
<td>41.7 ± 8.46</td>
</tr>
<tr>
<td>(2.90 - 4.34)</td>
<td>(224 - 663)</td>
<td>(15.7 - 29.6)</td>
<td>(2.6 - 7.6)</td>
<td>(34.6 - 51.8)</td>
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</tbody>
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TABLE II
PLASMA LEVEL ± S.D. OF THE 4 PROTEINS STUDIED AND OF RETINOL IN THE PCM GROUP (RANGE IN PARENTHESES AND PERCENTAGE OF CONTROL GROUP)

<table>
<thead>
<tr>
<th></th>
<th>Albumin (g/100 ml)</th>
<th>Transferrin (mg/100 ml)</th>
<th>TBPA (mg/100 ml)</th>
<th>RBP (mg/100 ml)</th>
<th>Retinol (μg/100 ml)</th>
</tr>
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<tbody>
<tr>
<td>Day 1</td>
<td>1.74 ± 0.39</td>
<td>1.31 ± 0.57</td>
<td>6.38 ± 1.89</td>
<td>1.62 ± 0.72</td>
<td>11.43 ± 4.26</td>
</tr>
<tr>
<td>Day 8</td>
<td>2.10 ± 0.46</td>
<td>2.14 ± 0.43</td>
<td>13.09 ± 3.79</td>
<td>3.53 ± 1.33</td>
<td>25.75 ± 9.91</td>
</tr>
<tr>
<td>Day 15</td>
<td>2.66 ± 0.41</td>
<td>343 ± 132</td>
<td>19.47 ± 5.38</td>
<td>4.94 ± 1.57</td>
<td>37.59 ± 9.52</td>
</tr>
<tr>
<td>Day 22</td>
<td>3.22 ± 0.37</td>
<td>445 ± 167</td>
<td>22.31 ± 3.47</td>
<td>5.41 ± 0.87</td>
<td>42.01 ± 9.71</td>
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<tr>
<td></td>
<td>52.8%</td>
<td>38.7%</td>
<td>28.5%</td>
<td>31.9%</td>
<td>27.4%</td>
</tr>
<tr>
<td></td>
<td>63.8%</td>
<td>63.3%</td>
<td>58.5%</td>
<td>69.6%</td>
<td>61.8%</td>
</tr>
<tr>
<td></td>
<td>80.8%</td>
<td>101.4%</td>
<td>87.1%</td>
<td>97.4%</td>
<td>90.6%</td>
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<tr>
<td></td>
<td>97.8%</td>
<td>131.3%</td>
<td>99.8%</td>
<td>106.7%</td>
<td>101.0%</td>
</tr>
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concentration averaging 10.3 ± 0.7 g/100 ml (range 8.1 to 12.8). In the malnourished group, the red blood cell count averaged on admission 3.15 × 10⁶ ± 0.43 × 10⁶/mm³ (range 2.3 × 10⁶ to 3.5 × 10⁶) and the blood hemoglobin level 8.1 ± 1.3 g/100 ml (range 6.2 to 11.2). After three weeks of dietary therapy, the hematological data had improved but were not normalized: 3.6 × 10⁶ ± 4.9 × 10⁶ RBC/mm³ (range 2.7 × 10⁶ to 4.1 × 10⁶) and 9.3 ± 1.7 g hemoglobin/100 ml (range 7.1 to 11.7).

Discussion

The comparative study of 4 plasma proteins reveals that in the acute stage of PCM, albumin responds with low sensitivity, transferrin with intermediate and the TBPA-RBP complex with the highest sensitivity. These findings are presumably the direct and main consequence of decreased liver biosynthesis. The return to normal of the albumin level is slow and regular, according to a classical and universally recognized pattern. The relative unresponsiveness of albumin to protein restriction makes its measurement inadequate for the detection of an early deficit. Estimation of the albumin level, however, is useful in manifest malnutrition [16].

Plasma transferrin appears to be more suitable in defining the nutritional status, since its decrease is more pronounced than albumin. In our survey, however, and as reported by others [4], plasma transferrin shows a wide dispersion of individual values on admission. In spite of the onset of severe kwashiorkor, some results are within the normal range. After the first week of dietary treatment, some individual values for transferrin increase by 6 or 8 times, as described earlier [1], whereas others remain unchanged or even may drop. Moreover, transferrin (and albumin) is regarded as a poor index for assessing the status of starving infants with marasmus [17,18]. This situation seems to arise from the peculiar behaviour of transferrin in alteration of iron metabolism: an
Fig. 1. Individual values of the 4 plasma proteins studied at different days of dietary therapy. The return to normal of albumin is slow and regular, whereas that of both components of the TBPA-RBP complex is fast and regular. Transferrin, as a whole, is characterized by an intermediate slope and sometimes unpredictable evolution of isolated values.

Elevation of transferrin levels is found in iron-deficient patients and a progressive return to normal values is observed following the onset of iron therapy [19]. In Senegal, nutritional anaemia is a common finding both in apparently healthy [20] and in protein-deprived children [21], the latter being more affected. The high values for transferrin obtained in our control group and in the group of recovering children on day 22 (0.01 > p > 0.001) suggests that iron deficiency plays a major role in the appearance of this nutritional anaemia. As predicted by previous works [19], the increase of blood transferrin reflects an attempted compensatory mechanism and might constitute a reliable index for assessing the severity of iron deficit. This situation, however, becomes manifest only when protein replenishment is achieved. Protein malnutrition
and iron deficiency are usually associated in patients living in developing countries, thus involving the occurrence of opposite stimuli upon transferrin biosynthesis. In other words, the actual plasma transferrin level expresses the relative predominance of one deficiency over the other. The coexistence of these contradictory impulses invalidates, at least in part, the measurement of transferrin as a dependable method for evaluating the protein status alone.

Results recorded for TBPA and RBP reveal the most pronounced decline, both on admission and during the subsequent steps of clinical recovery. Values obtained for TBPA and RBP seem to be negatively correlated with the extent of liver damage. Lower plasma concentrations for TBPA and RBP were found in children suffering from severe kwashiorkor and heavy liver steatosis (upper Wellcome class), whereas higher plasma levels were recorded in marasmic kwashiorkor with medium liver injury (lower Wellcome class). This finding is in agreement with recent data obtained in liver cirrhosis where prealbumin is regarded, among 21 different plasma proteins, as the most sensitive indicator of hepatic malfunction [22].

In comparison with albumin and transferrin, the lower values for TBPA and RBP in serum are at least in part the result of a faster turnover rate. In adult subjects, the biological half-life of albumin is about 20 days [23], and that of transferrin about 8 days [19] whereas this parameter for TBPA and RBP is estimated about 2 days [24,25] and 12 hours [26], respectively. The persistence of an unaffected molar ratio between TBPA, RBP and retinol during nutritional rehabilitation [9,10] suggests that, as in healthy adults, only the RBP molecules bound to TBPA remain in the blood stream. TBPA thus serves as a limiting factor for RBP binding, which in turn acts as the limiting factor for retinol transport. Environmental conditions that restrict protein intake (or protein assimilation) seem to involve repercussions in liver, depressed TBPA biosynthesis and reduced serum RBP-retinol levels. The return of retinol to normal plasma levels during nutritional rehabilitation mainly results from its liver release rather than from the dietary intake [10].

Tryptophan is known to exert a key role in the regulation of protein synthesis [27,28]. Tryptophan is practically undetectable in the blood of PCM children [29], and its catabolite spectrum implies profoundly disturbed pathways [6]. The combination of a very rapid turnover rate and an exceptionally high tryptophan content presumably explains why TBPA and RBP respond with marked sensitivity to protein shortage. Furthermore, it is our clinical experience in Senegal that TBPA is the first blood protein to be significantly decreased, as a result of borderline protein intake, in apparently healthy children (unpublished data). This peculiar behaviour allows the identification of all prekwashiorkor stages and also of all early forms of marasmus, even when the other available tests still lie within the normal range. Measurement of TBPA is, therefore, ideally suited for the screening of preschool children and for field studies.

On the basis of the parallel evolution of TBPA, RBP and retinol, diminished values for vitamin A levels reported in low-income people [30] could reflect a chronic imbalance in protein diet. Moreover, seasonal variations in signs of vitamin A-deficiency at the very time when dietary supply of vitamin A remains unchanged [31] could be related to temporarily reduced protein intake.

The presented data imply that each of the 3 molecules constituting the
retinol circulating complex might, theoretically, be useful in estimating protein depletion. Measurement of retinol, however, is not possible under field conditions. Moreover, since blood concentration of RBP is 4 or 5 times lower than that of TBPA, we suggest that the evaluation of serum prealbumin level as compared to albumin and transferrin, would be at the present time the most useful and accurate index in defining nutritional status.

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