Plasma and urine ribonuclease as a measure of nutritional status in children[1, 2, 3]

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As is common in most diseases, early emphasis in protein–calorie malnutrition of childhood has focused on the most severe forms. This is because both frank kwashiorkor and third degree marasmus are easily recognized, dramatic in their appearance, and serious in their prognosis. This focus has led to an emphasis on the corrective, rather than on the preventive, aspects of the disease. More recently, as the prevalence of less severe malnutrition has been realized and the possible serious consequences understood, interest has developed in milder forms of undernutrition. However, in grade I or II malnutrition, accurate diagnosis has proved difficult. For this reason, a number of people have been seeking a biochemical marker for nutritional status in less severe types of childhood undernutrition. Establishment of such a marker would also allow us to diagnose protein–calorie malnutrition in its early stages and to focus our attention on prevention rather than on treatment. Our goal was to find a biochemical marker for nutritional status that is reliable, easily performed, and practical under field conditions in cases of even mild undernutrition.

Alkaline ribonuclease (RNase) activity is elevated in a number of rat tissues after a period of malnutrition. Neonatal undernutrition markedly increases the activity of this enzyme in rat brain (1). RNase activity is increased in placentas in cases of vascular insufficiency in the rat (2) and maternal malnutrition in the human (3); these findings suggest that RNase may be a marker for tissue malnutrition. Therefore, as this enzyme is measurable in blood (4) and is excreted in urine (5), we have measured its activity in these fluids in normal adults, in children of various ages, and in malnourished infants, with grade I, II, and III malnutrition. In addition, in the children with grade III malnutrition, we have measured enzyme activity after 2 weeks of rehabilitation.

Materials and methods

Subjects of the study

Blood and urine samples were obtained from 48 normal individuals: 38 infants from 0 to 14 months of age and 10 adults. Ten premature infants between 4 and 57 days of age, weighing 1,800 to

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[2] Supported by National Foundation Grant CRBS-262 and Public Health Service Grant HD03888 from the National Institutes of Health, Bethesda, Maryland 20014.

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2,200 g, who had reached a stable condition and were on a full diet and growing normally, were also studied.

Thirty-four malnourished children from 2 to 48 months of age were studied: 10 with grade I or II malnutrition and 24 with grade III malnutrition (marasmus). In 10 cases with grade III marasmus, follow-up studies were performed after 15 days of rehabilitation. All children were characterized according to the Gomez classification of nutritional status (6) by two pediatricians. Children with infectious diseases or evidence of iron or vitamin deficiencies were excluded. Although the children were not specifically selected as to economic status of the family, but were chosen randomly from the populations being serviced at local health clinics in Sao Paulo, Brazil, and Santiago, Chile, most came from a poverty stricken background and had historical evidence of both protein and caloric deficiencies. The children did not show signs or symptoms that characterize kwashiorkor; total serum protein levels were within the normal range. In addition, 13 full-term, singleton, small-for-date infants from uncomplicated pregnancies, weighing between 2,000 and 2,500 g were studied.

Methods

When blood samples were anticoagulated, 0.1 M EDTA was added in the amount of 0.1 the sample volume. Serum or plasma was separated by centrifugation for 20 min at 200 *g*, saved in plastic tubes, and kept frozen until day of assay.

The first urine sample voided after the blood collection was studied. The urine samples were centrifuged for 20 min at 200 *g*, and the clear supernatant was saved in plastic tubes and kept frozen until assay.

Free alkaline (pH 7.8) RNase activity was determined by a modification of the method of Roth (7). The assay mixture contained 0.3 ml freshly diluted 0.07 M veronal acetate buffer, 0.2 ml RNA substrate (1% solution of grade A, highly polymerized yeast RNA, Calbiochem, Los Angeles, California), and 0.1 ml diluted sample. The plasma samples were diluted to 50% and the urine samples to 25% of their original concentrations with the veronal acetate buffer before assay. Samples were incubated for 30 min at 37 C and the reaction stopped by the addition of 0.6 ml of cold acid–alcohol–lanthanum chloride reagent prepared according to Roth (7).

Following 20 min centrifugation at 1,000 *g* in the cold, the supernatant from samples and their zero time controls were decanted, diluted 1:10 with distilled water and read against distilled water at 260 mμ in a Beckman DU-2 spectrophotometer. Units of RNase activity were arbitrarily defined as:

$$\text{Optical density of sample} - \text{optical density of zero time} \times 100.$$

The data were ultimately expressed in units per milliliter.

Total protein in plasma was determined by the biuret method (8). Creatinine in plasma and urine was determined by the Jaffe reaction (9).

Results

In normal individuals, free alkaline RNase activity in plasma is significantly higher at or shortly after birth than thereafter ($P < 0.001$) (Table 1). Although the exact time the drop occurs is not known, it is at some point between 10 and 20 days after birth. By 20 days of age, enzyme activity in normal full-term infants has dropped to adult levels. Plasma values in 13 small-for-date newborn infants (13,142 ± 2,063) and 10 prematures (11,125 ± 2,034) were not different from the normal full-term levels (12,118 ± 1,971), even though some prematures were approximately 2 months of age.

In contrast, the free RNase activity in plasma in all malnourished infants was significantly elevated when compared with 16 age-matched, well-nourished control children ($P < 0.001$) (Table 2). After only 2 weeks of

TABLE 1
Free RNase activity in plasma or serum of normal individuals

Age	Units of RNase/ml
0 days (12)[a]	11,712 ± 1,393
1–10 days (10)	12,118 ± 1,971
20 days–14 months (16)	5,756 ± 1,058
19–37 years (10)	5,687 ± 706

[a] Number in parentheses represents sample size.

TABLE 2
Free RNase activity (in units per milliliter)

Malnutrition			Normal
Grade I and II	Grade III	Grade III (refed)	
8,422[a] ±937 (10)[c]	9,428[a] ±1,881 (24)	6,391[b] ±1,139 (10)	5,756 ±1,058 (16)

[a] $P < 0.001$. [b] NS. [c] Number in parentheses represents sample size.

rehabilitation, levels returned to normal. Note that the levels in the 10 patients with less severe grade I or II malnutrition were no different from the values in the more severely malnourished children. Thus, postnatal malnutrition of even a mild degree causes an elevation of plasma RNase activity.

Mean free RNase activity per milliliter of random urine samples in normal individuals is greater in adults and infants over 7 months of age than in infants under 7 months ($P < 0.001$) (Table 3). Infants older than 7 months with grade III malnutrition had lower mean activity than infants of the same age with grade I or II malnutrition, but the differences were significant only at the level of $P < 0.01$. The extremely wide variation in the data make discrimination between individual patients on the basis of an isolated urinary value impossible. Urine levels were also calculated per milligram creatinine with no significant improvement in the scatter of the data and therefore have not been reported here.

The urine/plasma ratio for free RNase activity also was calculated. The data demonstrate that the ratio increases significantly in the second 6 months of the 1st year of life ($P < 0.001$) (Table 4). The small, further increase in adult levels is not significantly above the 7 to 14 months value. There is a marked reduction in the urine/plasma ratio in infants with grade III malnutrition. Values in these children, regardless of their age, were comparable to the low levels seen in normal infants under 7 months of age. By contrast, in the six children over 7 months of age with grade I and II malnutrition, urine/plasma ratios were in the same range as their age-matched controls. Thus, their values were significantly higher than values in any of the children with grade III malnutrition ($P < 0.001$).

In summary, it was possible to discriminate between normal and malnourished children by measuring free RNase activity in plasma. The additional calculation of urine/plasma ratios helped to distinguish between grade III and less severe degrees of malnutrition.

TABLE 3
Free RNase activity in urine

Group	Age	Units of RNase/ml
Normal	2–7 months (8)[a]	4,511 ±1,489
	7–14 months (16)	19,823 ±6,037
	10–37 years (10)	24,278 ±7,684
Grade I and II malnutrition	7–14 months (6)	32,743 ±9,175
Grade III malnutrition (marasmus)	2–7 months (6)	10,483 ±8,688
	7–48 months (11)	15,193 ±12,828

[a] Number in parentheses represents sample size.

TABLE 4
Urine/plasma RNase ratios

Group	Age	Urine/plasma
Normal	2–7 months (8)[a]	0.86 ± 0.31
	7–14 months (5)	3.62 ± 0.58
	19–37 years (10)	4.30 ± 1.43
Grade I and II malnutrition	7–14 months (6)	3.70 ± 0.85
Grade III malnutrition (marasmus)	2–7 months (3)	1.00 ± 0.56
	7–48 months (9)	1.25 ± 0.44

[a] Number in parentheses represents sample size.

Discussion

By measuring free RNase activity in plasma and urine, we have been able to separate normal infants and children from infants and children with different degrees of malnutrition. Figure 1 summarizes the data; urine/plasma ratio is plotted against plasma RNase for four study groups. Note that plasma levels are equal in normal children regardless of age. By contrast, children over 7 months have a mean urine/plasma ratio above 3.0, whereas younger infants have a ratio of approximately 1.0. All malnourished children have elevated plasma RNase levels (>7,500 units/ml). However, the less severely ill children have normal urine/plasma ratios, whereas the severely marasmic infants have

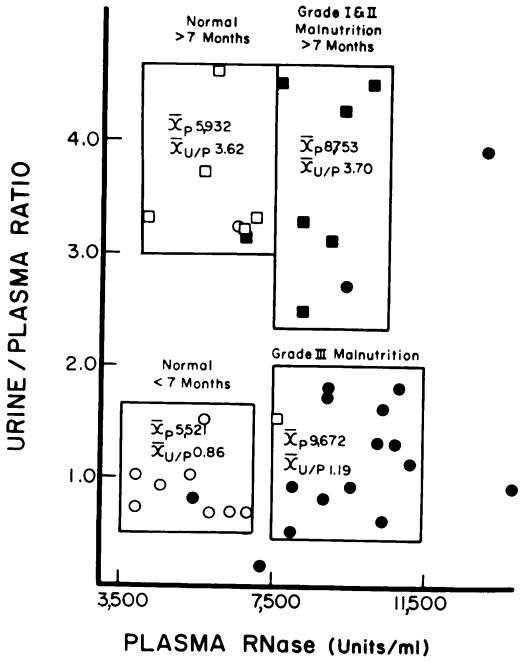

FIG. 1. Plasma levels are equal in normal children regardless of age. Children over 7 months have a mean urine/plasma ratio above 3.0, whereas younger infants have a ratio of approximately 1.0. All malnourished children have elevated plasma RNase levels. The less severely ill children have normal urine/plasma ratios, whereas the severely marasmic infants have a low urine/plasma ratio. The means for each group are noted in each of the boxes that have been drawn free-hand in order to encompass the majority of points for each group. Open squares represent normal infants 7 to 14 months of age; open circles, normal infants from 1 to 7 months of age. Closed squares represent infants from 7 to 14 months of age with grade I and II malnutrition; closed circles, infants from 2 to 48 months with grade III malnutrition.

a low urine/plasma ratio. This reduction is due to a decreased urine RNase concentration. This is consistent with the findings of Donoso et al. (10) who have documented increased free water clearance and decreased concentrating ability in such patients. In the patients over 7 months of age with grade I and II malnutrition, urine/plasma ratios were normal for their age. This is because these less severely malnourished children have retained their ability to concentrate their urine, resulting in an excretion of RNase that is greater than normal in random urines.

The clearance of plasma RNase is due, at least in part, to renal excretion. Serum RNase activity increases after bilateral nephrectomy in the rat (5) or when the glomerular filtration rate is decreased in man (11). However, marasmic patients do not have a reduction in glomerular filtration rate (10), and measurements of plasma creatinine in our cases showed no difference between the malnourished (0.44 ± 0.10 mg/100 ml) and control (0.40 ± 0.20 mg/100 ml) groups. Animal studies (12) have revealed an elevation of tissue RNase after malnutrition and one might speculate that the increased tissue levels are reflected in the plasma. However, no tissue levels of RNase have been carried out in the malnourished human and the intracellular metabolism or degradation of RNase has not been determined. Therefore, the functional alterations leading to the elevation of plasma RNase in malnutrition are, at present, unknown.

Plasma RNase would seem to be the best parameter for discriminating between normal and malnourished children. Additional comparison of urine/plasma ratios should then assist in distinguishing between grade III malnutrition and less severe malnutrition. Because measurement of free alkaline RNase activity is not difficult, and as it is relatively stable in stored samples of blood and urine, additional investigation of its usefulness in field studies is warranted and it is expected that such studies will be rewarding.

Summary

In the present study, free alkaline RNase was measured in plasma and urine of normal and malnourished children. Levels of this enzyme in plasma and the urine/plasma ratio make it possible to discriminate between normal and malnourished children with even a mild degree of malnutrition.

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