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Serum Tocopherol Levels of Normal Preschool Children and Children with Protein-Calorie Malnutrition in South India* (32628)

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Majaj (1) has drawn attention to the possibility that vitamin E responsive anemias may occur in association with protein–calorie malnutrition. This has created considerable interest in the investigation of the causes of anemias associated with protein–calorie malnutrition which occur in various parts of the world. Data have been reported on the concentration of α-tocopherol in the serum of normal children above 5 and below 2 years of age (2,3,4). However, no data on the α-tocopherol content of the serum of normal preschool children between 2 and 5 years of age has been reported (preschool years show the highest incidence of protein-calorie malnutrition). For this reason α-tocopherol has been determined in normal South Indian children between 2 and 5 years of age and in children with kwashiorkor. Two groups of healthy preschool children have been studied, a group from a typical South Indian village and a group from an orphanage. The α-tocopherol in the serum of these healthy children has been compared to that found in patients with kwashiorkor, most of whom come from similar villages. The anemias occurring in patients with kwashiorkor are also under investigation.

Materials and Methods. Forty-two children aged 1–4 years with the clinical signs of protein–calorie malnutrition (5), admitted to the Nutrition Research Unit of the Christian Medical College and Hospital, Vellore, took part in the study.

The patients were fed a diet which provided 3 gm protein per kg per day from skimmed milk, and 150 cal/kg/day from rice, sugar, and coconut oil (6). Calories from oil formed 15% of the daily intake. The patients were given 10,000 IU of vitamin A in 1 cc peanut oil daily as part of their therapy. This diet provided about 3 mg α-tocopherol/day, calcu-

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TABLE I. Average Daily Intake of Proximate Principles and Tocopherol.

	Protein[a]	Fat[a]	Calories[a]	α-Tocopherol (mg)[b]
Children with kwashiorkor				
In hospital	22.5	19.00	1125	3.0
Normal subjects				
At orphanage	20.0	13.3	950	3.70
At creche	20.0	12.07	1004	3.78

[a] Computed from standard Indian food tables.
[b] Computed from Engel (7), Bunnell *et al.* (8) and Dicks (9).

lated with reference to Engel (7), Bunnell *et al.* (8), and Dicks (9).

The normal subjects were 99 children, 2–5 years of age. Fifty-seven were resident in an orphanage and 42 attended a day-care center in a village (creche).

The normal subjects had a diet similar in composition but somewhat higher in quantity to that eaten in the surrounding villages. The cereals were rice and ragi (*Eleusine coracana*), eaten with small amounts of legumes and vegetables. The diets contained no animal products and were prepared from standardized recipes. The cooking oil in both the orphanage and at the creche was peanut oil. The composition of these diets has been reported in detail elsewhere (10,11). In both centers, the diets provided the children with 2 gm vegetable protein and 100 cal per kg body weight per day. In the orphanage this formed the entire dietary intake. In the village, the children ate snacks in their homes which provided about 100–200 calories/day in addition to the food eaten at the creche.

Blood was drawn by venepuncture from fasting subjects. The samples were collected in stoppered centrifuge tubes on ice in insulated containers. Serum was separated and frozen under nitrogen; the samples were analyzed within 1 week.

A slight modification of the Emerie-Engel reaction for the analysis of vitamin E described by Bieri *et al.* (2) was employed. One-half ml of serum was mixed thoroughly with 2.0 ml of 95% ethanol in a glass-stoppered centrifuge tube. Three ml of *n*-hexane were added followed by 1 ml of distilled water. Before replacing the stopper, a current of nitrogen was passed into the tubes. The centrifuge tubes were placed in a mechanical shaker for 30 min and then centrifuged for 5 min at 1500 rpm. Two ml of the supernatant were pipetted off into a small glass tube and the solvent was evaporated under nitrogen in a waterbath at 60°C. One-tenth ml of redistilled chloroform was added to the residue followed by 1 ml of ethanol, 0.1 ml of 0.2% $\alpha\alpha'$-dipyridyl and 0.1 ml of 0.1% ferric chloride reagent. The tube was shaken vigorously for 10 sec. The contents were poured into the Beckman microcuvets and the optical density was read at 520 mμ exactly 40 sec after the ferric chloride reagent was added. Standard curves were drawn using *dl*-α-tocopherol.

The concentration of vitamin A was also estimated in these same sera in connection with another study which has been previously reported (12). No carotenoids were present in these sera, based on the absence of any significant absorption at 452 mμ of the petroleum ether extracts. Consequently no correction for interference due to carotenoids was necessary in the estimation of α-tocopherol.

Ethanol was purified by treatment with NaOH and repeated distillation until yellow coloration did not appear on the addition of NaOH. *n*-Hexane was shaken with saturated $KMnO_4$, washed with dilute 0.1 *N* sulfuric acid, and repeatedly washed with water until all traces of acid were removed.

Thin-layer chromatographic separation of the tocopherols was carried out using the Bieri (13) method in 6 normal subjects. Serum cholesterol levels were determined by the Carr and Drekter (14) method in 17 children with kwashiorkor and 55 normal subjects.

Results. Table I gives the daily tocopherol intake of the children. Serum tocopherol

TABLE II. Serum Tocopherol Values of Normal Subjects and Children with Kwashiorkor.

Subjects	Serum tocopherol (μg/100 ml) Mean ± SD[a]	Range
42 children with protein-calorie malnutrition	410.8 ± 184.5	120- 870
57 children at orphanage	649.9 ± 177.7	285-1110
42 children at creche	470.7 ± 112.3	258- 686

[a] One standard deviation.

values of children with protein–calorie malnutrition and those of the normal subjects at the orphanage and at the creche are presented in Table II.

Twenty-two percent of the children in the orphanage had serum values below 500 μg/100 ml, while 43% of the children at the creche had similar values. None of the children at the creche had values above 600 μg/100 ml whereas 36 (63%) of the children in the orphanage had values above this figure.

The average tocopherol values of the patients with kwashiorkor on admission to the hospital was 410.8 μg and the range 120–870 μg/100 ml. Twelve patients had values below 300 μg/100 ml and 18 values were between 300 and 500 μg/100 ml. The remaining 12 patients had levels above 500 μg/100 ml including 3 above 800 μg/100 ml.

With regard to the normal subjects, there was a significant difference (Students t test, $p<.1\%$) in the tocopherol levels of the children in the orphanage compared with the children at the creche, although the computed daily intake of tocopherol was the same. All children on whom data is reported were receiving weighed amounts of diets of known composition. The α-tocopherol intake was computed from values in Standard Indian food tables and in Engel (7), Bunnell *et al.* (8), and Dicks (9).

The children with protein–calorie malnutrition had values similar to those of the children attending the creche, but lower than those of the children at the orphanage.

Serum cholesterol values of the normal children and of the patients are presented in Table III. The children with kwashiorkor had a mean cholesterol level of 78 with a range of 50–206 mg/100 ml. The serum cholesterol values of the children at the orphanage were higher than those of the children from the creche. The mean serum cholesterol level of the children from the orphanage was 135 mg with a range of 81–193 mg/100 ml and that of the children from the creche was 88 mg with a range of 50–133 mg/100 ml. The difference between the two groups was statistically significant ($p<.001$).

The changes in serum α-tocopherol values of children with protein–calorie malnutrition are presented in Table IV. On admission their serum α-tocopherol values had a mean of 375 μg/100 ml. There was a significant rise ($p<.01$) to a mean of 575 μg/100 ml in a period of 2 weeks on a diet providing about 3 mg of α-tocopherol per day.

Discussion. According to the reports of Guzman *et al.* (15), school children from 5 Central American countries had an average tocopherol value of 650 μg/100 ml. Krukowa (4) observed a level of 850 μg/100 ml in 15 children, aged 1–2 years, in Poland. In an East Pakistan survey, 30% of 31 children, aged 6–14 years, had α-tocopherol levels below 500 μg/100 ml (3).

The data presented in this paper refer to

TABLE III. Serum Cholesterol Values of Normal Subjects and Children with Kwashiorkor.

Subjects	Serum cholesterol (mg/100 ml) Mean ± SD[a]	Range
17 children with protein-calorie malnuitrition	78 ± 8.97	50-206
40 children in orphanage	135 ± 29.5	81-193
15 children in creche	88 ± 25.5	50-133

[a] One standard deviation.

TABLE IV. Changes in Serum Tocopherol in Patients on a Diet Providing 3 mg Vitamin E per Child per Day.

Time	Serum tocopherol (μg/100 ml) Mean ± SD[a]	Range
On admission	375.5 ± 185.0	180-690
After 2 weeks	575.3 ± 251.7	330-885

[a] One standard deviation.

the tocopherol levels of children aged 2–5 years. In 99 normal children, the average value was 574 μg/100 ml and 75% of the children had values between 400–800 μg/100 ml.

The children in the orphanage had higher serum values than the children at the creche. Both groups of children were of the same age. Estimation of hemoglobin, serum vitamin A, and serum proteins showed no difference between the two groups. Their diets supplied similar amounts of fats and the cooking medium (peanut oil) was the same.

One of the differences between the two groups of normal children was that the children at the creche, in addition to the diet supplied ate snacks in their homes. The additional food accounted for an extra 100–200 calories per child and was composed of cerals and oils such as peanut and sesame oil. Despite this higher intake, their α-tocopherol levels in the serum were low when compared to the children at the orphanage. No explanation for this difference in the serum tocopherol values is readily available, except perhaps that the children at the creche were more active than those at the orphanage.

The residents at the orphanage had higher cholesterol values than the children at the creche, again, despite similar intakes of the same type of fat.

The children at the orphanage, taken as a group, had high cholesterol and high α-tocopherol values. However, there was no correlation between the higher α-tocopherol values and high cholesterol values when individuals were studied.

In an average American diet, Bunnell *et al.* (8) have calculated that the daily intake of α-tocopherol ranges from 2.4 to 15.4 mg with an avearge of 7.4 mg. Horwitt (16) stated that as little as 5 mg of α-tocopherol a day is adequate for an adult on a diet low in unsaturated lipids. However, prolonged ingestion of large amounts of unsaturated fat may demand a higher vitamin E requirement. In normal preschool children both from the orphanage and the day creche, the intake of fat (about 13 gm day) and therefore of polyunsaturated lipids was low. For these subjects 3–4 mg of dietary tocopherol, as calculated from their daily intake, appeared to be enough to maintain normal serum levels in most of the children.

It is evident from the data presented that the majority of South Indian children studied had higher levels of tocopherol in their serum than were reported by Majaj (1,17) in the Jordanian children in whom vitamin E responsive anemias have been studied. However, in 12 of our patients with protein–calorie malnutrition, vitamin E levels below 300 μg/100 ml were recorded.

In previously reported studies from this laboratory on the anemias of patients with kwashiorkor (18) the great majority were associated with the low levels of serum folate and responded to therapy with folic acid or vitamin B_{12}. Studies on these anemias are continuing and will be reported with reference to the possible role of vitamin E.

The low levels of α-tocopherol reported by Asfour (19) (using Quaife's micromethod of vitamin E determination) were not encountered in our series of children with protein–calorie malnutrition who had serum vitamin E levels that were within the lower limits of normal when compared with normal subjects. Within 2 weeks of hospitalization and on a diet that provided 3 mg/day of tocopherol, serum vitamin E levels rose substantially. It may be significant that coconut oil was the main source of fat in the hospital diets of these patients in contrast to the peanut oil used in the diets of the normal subjects. Despite the impaired digestive powers of the patients, absorption of vitamin E was apparently adequate.

Summary. Serum tocopherol levels of 99 normal children, aged 2–5 years, and of 42 children with kwashiorkor were estimated. Of the 99 children, 57 from an orphanage had an average serum tocopherol value of 649 μg/100 ml whereas 42 children from a day-care creche had an average level of 470 μg/100 ml. The patients with kwashiorkor had an average value of 410 μg/100 ml when admitted to the hospital. The estimated dietary intake of α-tocopherol of the normal preschool children was 4 mg per child per day.

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Thyrocalcitonin Suppression of Hydroxyproline Release from Bone*† (32629)

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It is now generally accepted that the hypocalcemic effect of thyrocalcitonin is mediated primarily by a reduction in the rate of calcium removal from bone (1–5). However, as is still true with parathyroid hormone, the actual mechanism by which this recently discovered hormone accomplishes its effect is still to be elucidated. In most aspects studied, thyrocalcitonin has been shown to be antagonistic to parathyroid hormone, but its action is independent as it is fully effective in parathyroidectomized animals (6). A relevant current controversy concerning parathyroid hormone action on bone is related to its ability to increase the resorption of all components of bone: apatite crystal, collagen and the mucopolysaccharide ground substance. One can not say with certainty whether the hormone affects all intercellular bone components concurrently, or whether the action on one component is primary and those on the other components are produced indirectly.

At the present time, thyrocalcitonin action on bone has been studied briefly in regard to its ability to decrease the transfer of calcium and phosphate from bone to extracellular fluid. It is the purpose of this report to examine its effect on collagen breakdown and to determine whether thyrocalcitonin is antagonistic to the action of parathyroid hormone on this process. Friedman and Raisz (7), Gaillard (8) and Aliapoulios *et al.* (9) have reported that, in *in vitro* systems, thyrocalcitonin can block effects normally produced by

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