Lysine Supplementation of a Rice and Milk Diet Fed to Children Recovering from Kwashiorkor and Undernutrition

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DIETS consumed by a majority of the population in Ceylon are deficient in calories and in proteins,[1-3] especially protein of high biologic value. The staple food in Ceylon is rice which, like most cereals, is relatively deficient in some indispensable amino acids, chiefly lysine.[4,5] While there is a large volume of evidence to indicate that supplementation of wheat flour with lysine results in a product of increased biologic value,[6-8] similar supplementation of other cereal foods has yielded equivocal results. Zein, even when supplemented with theoretically adequate levels of the essential amino acids in which it is deficient, does not support optimum growth of rats.[9-11] A slight but significant improvement in white rice has been reported when it is supplemented with methionine,[12] with lysine[12,13] and with lysine and threonine[5]; in many of these experiments, however, the diets contained purified constituents or consisted primarily of one staple food, for example, rice. Baptist and de Mel,[14] on the other hand, found that a rice and legume diet was capable of supporting growth in healthy children, but the rate of growth in rats maintained on similar diets has been slower and smaller than in those on a stock diet.[15,16] Gomez and co-workers[17] found that the absorption of nitrogen from a diet of maize and beans by children suffering from kwashiorkor was low, but could be significantly increased by the addition of milk. Albanese et al.,[18] on the other hand, found it was necessary to add lysine to a diet consisting mainly of milk to bring about optimal growth.

This is a report of the results of a study of lysine supplementation of a diet fed to children between the ages of nine months and two years recovering from general undernutrition and from kwashiorkor at the Lady Ridgeway Children's Hospital, Colombo, Ceylon, during the period June 1957 to February 1958. The problem of supplementation of rice diets consumed by children in Ceylon is briefly discussed.

EXPERIMENTAL STUDIES

The differentiation between general undernutrition and kwashiorkor was made on criteria described previously.[19] As far as possible children of similar age were selected for the different groups. Eight children recovering from general undernutrition were divided into two groups, one to be given lysine (group 1), the other (group 2) being kept as controls. Owing to an insufficiency of numbers, all patients recovering from kwashiorkor (a total of five) were grouped together (group 3) and given the supplement.

Each child was treated for one to three weeks until the clinical condition improved, and was then given a diet consisting of some milk and fruit (see Table 1). After a period of thirty days on this diet (period 1) lysine in the form of L-lysine hydrochloride (supplied by

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TABLE 1
Plan of Diet Fed to the Children

Morning meal
Milk
Bread and butter
Mid-morning drink
Orange juice
Lunch
Rice, leafy vegetable,* other vegetable† or milk with rusks or bread
Banana
Afternoon tea
Bread or rusks with banana
Dinner
As for lunch
Late night drink
Malted milk

NOTE: The vegetables are cooked in coconut milk which is obtained by mixing finely grated coconut kernel with water and expressing the "milk" by hand. The cooked "milk" was also served as gravy. During experimental period 2, lysine (50 mg. of L-lysine hydrochloride per lb. body weight) was given with the milk fed to children in groups 1 and 3.

* One of the following: mukunuwenna (Alternanthera triandra), spinach (Basella alba), tampala (Amaranthus var.), agati leaf (Sesbania grandiflora).

† One of the following: lentil (Lens esculenta), ash plantain (Musa sepientum), snake gourd (Trichosanthes anguina), okra (Hibiscus esculentus).

the E. I. du Pont de Nemours Co., Inc.) was added to the diet of groups 1 and 3 (50 mg. per pound body weight of the hydrochloride being mixed with the milk each day). The administration of lysine was discontinued after thirty days (period 2) and the unsupplemented diet was then given to all three groups for a further period of thirty days (period 3). The children were allowed to eat as much as they desired and the amount of food consumed was measured. Vitamin supplements were given to all the children throughout the experiment.

At weekly intervals a clinical assessment of each child was made, measurements of height, weight and thickness of subcutaneous tissue were taken, and blood was drawn for the estimation of plasma proteins.

Subcutaneous Tissue Measurement

A pair of Harpendon skinfold calipers (British Indicators, Ltd.) recommended by Edwards et al.[20] was used, the readings being taken by the same observer on all the children throughout the experiment. Two readings were recorded over the inferior angle of scapula on each side at minute intervals and the mean value regarded as a criterion of fatness.

Plasma Proteins

Heparinised venous blood was centrifuged, the proteins in 0.2 ml. of the separated plasma were precipitated with tungstic acid, and the nitrogen was estimated by the microKjeldhal method. The plasma proteins were fractionated by filter paper electrophoresis using Whatman No. 1 filter paper strips (3 cm. by 25 cm., previously soaked in 1 per cent sodium citrate and dried) and veronal buffer, pH 8.6, a voltage of 110 volts being applied for sixteen hours. The dried paper was stained in amido-black 10B,[21] rendered translucent and scanned in densitometer.

The phospholipids in plasma were estimated in a few cases by the extraction of a trichloracetic acid precipitate of the plasma with an ethanol-chloroform-ether mixture previously used.[22]

RESULTS

During the latter half of the third experimental period three children contracted measles, two had infective diarrhoea and one acute bronchitis. Therefore, the data obtained during the last thirty days of the experiment are not presented.

Food Consumption

The weights of cooked foods consumed were converted to dry weight and the calorie and protein content calculated from published data.[23] The lysine and threonine contents of the proteins were estimated from values published by Block and Weiss.[24]

During the course of the experiment the children were gradually weaned from the almost total milk diet given for the intiation of cure to one containing bread, rice and vegetables as well as milk. They were encouraged to eat as much rice or bread as possible before the milk was offered them. The amount of calories, proteins, lysine and threonine derived from the diet by the children in the different experimental groups during the four weeks preceding (period 1) and during the supple-

TABLE II

Average Daily Intake (per kg. Body Weight) of Calories, Proteins, Lysine, and Threonine, and the Change in Body Weight (as gm. per Day) of the Children During the Four Weeks Immediately Preceding (Period 1) and During (Period 2) Lysine Supplementation

Group	Period	Calories	Proteins (gm.)	Lysine (mg.)	Threonine (mg.)	Change in Body Weight (gm. per day)
1	1	58	2.7	183	123	36
	2	67	2.5	154	101	24
2	1	57	2.6	169	114	32
	2	63	2.0	107	76	27
3A	1	61	3.1	204	137	25
	2	72	2.4	156	98	22
3B	1	63	2.2	183	95	− 7
	2	74	1.9	121	79	28

mentation of the diet with lysine (period 2) is given in Table II. The amount of food consumed varied considerably among the different subjects and also in the same subject during different weeks. There was a slight drop in food consumption during the first week of lysine supplementation in many cases, but these children soon recovered their appetite as supplementation was continued. The addition of lysine did not result in a marked improvement in appetite as noticed by Albanese et al.[18]

The calorie intakes were well below the value of 110 calories per kg. body weight recommended for children between six months and three years of age,[25] and agree with the values obtained by Pretorius and Smit[26] during the treatment of patients with kwashiorkor. The protein intakes were in accord with values of 2.2 and 3 gm. per kg. body weight suggested by György and by Holt.[27,28] The amounts of lysine and threonine taken by these convalescent children, who were replenishing depleted tissues, were equal to those recommended by Albanese[8] for normal children. Our results indicate that the amounts recommended by Pratt[29] and by Holt and Snyderman,[30] namely, 90 mg. lysine and 60 mg. threonine per kg. body weight, are more likely to represent the requirements of normal children in this age group.

Growth

The change in body weight of the children during the first two experimental periods is shown in Table II. The group of patients with kwashiorkor has been sub-divided into groups 3A and 3B as two of these subjects showed an increased rate of growth during period 2.

The rate of change of body weight and of "body fat" (as measured by the calipers) was not increased by lysine supplementation. The rates in groups 1 and 3A were similar to those for group 2 which received no supplement. Only the two children in group 3B grew more rapidly during lysine supplementation than before. It is possible that the fall in weight of these children during period 1 was due to the continued loss of water from the tissues during recovery from acute kwashiorkor, even though no oedema was apparent clinically at the commencement of the experiment. This was borne out by corresponding changes in the subcutaneous tissue measurement.

Measurement of subcutaneous tissue has been widely used in the assessment of human nutrition;[31] measurement over several sites is recommended: the triceps, subscapular and the supra-iliac sites[20] being considered to give the most accurate assessment of subcutaneous fat in any person at a given time. However, measurement over the same site during successive periods of time gives a reliable indication of a change in the rate of growth, as shown by results obtained in this experiment and also by valves obtained on healthy children of the same age group.[32] Changes in subcutaneous tissue measurement in general paralleled changes in body weight, a fall in weight being accompanied by a decrease in the reading.

TABLE III
Plasma Protein Concentrations (in gm. per 100 ml.) During the Three Weeks Prior to and During Lysine Supplementation of the Diet

Group	Period	Total Protein	Albumin	Alpha$_1$-globulin	Alpha$_2$-globulin	Beta-globulin	Fibrinogen plus Gamma Globulin	Albumin: Globulin Ratio
1	1	5.78	2.81	0.21	0.62	0.67	1.53	0.99
	2	6.39	3.34	0.18	0.59	0.78	1.48	1.10
2	1	5.63	2.70	0.20	0.55	0.59	1.64	0.97
	2	6.44	3.15	0.23	0.60	0.72	1.75	0.98
3A	1	6.31	3.44	0.19	0.54	0.69	1.74	0.99
	2	6.64	3.50	0.16	0.70	0.64	1.77	1.15
3B	1	5.92	3.00	0.25	0.49	0.60	1.60	1.04
	2	6.37	3.20	0.21	0.61	0.83	1.56	1.01

NOTE: Three estimations were made on each child during each experimental period.

Plasma Proteins

The concentration of proteins in blood plasma also gives an indication of the state of nutrition of an individual.[31] The results obtained in this experiment are given in Table III. The values obtained during the three weeks preceding lysine supplementation and during the first three weeks of period 2 have been averaged for each child and the mean for the group obtained.

During period 1 the total proteins and the albumin fraction increased rapidly in all the groups, but the rate of increase diminished markedly in period 2. The changes in groups 1 and 3 must be due to a gradual return of the child to normality and not to the administration of lysine, as similar changes were seen in group 2. The other protein fractions showed no marked change during the experiment. No significant fall in the alpha-globulin level was seen with recovery from kwashiorkor, as has been reported by others,[33,34] probably due to the fact that the children had recovered from the acute stage before they were selected for the experiment.[35]

There was an initial rise in plasma phospholipids in patients with kwashiorkor and in those with general undernutrition. The mean value for phospholipid phosphorus per 100 milliliters plasma fell from 13.4 mg. at the start of the experiment to 8.3 mg. at the beginning of period 2 and was 7.4 mg. at the beginning of period 3. Similar changes in serum bound lipids have been reported by Dean and Schwartz[36] in patients with kwashiorkor and are likely to be due to a sudden increase in the rate of growth which had been halted under the stress of malnutrition.[37] Lysine supplementation had no effect on serum phospholipids.

COMMENTS

The number of children in each group is too small for a statistical analysis of the results. From our data, however, it may be concluded that the addition of lysine to the diet fed to these children did not bring about an increased rate of growth. The children maintained a steady improvement in weight gain during the experimental periods, and the rate of growth of those given lysine was similar to that of the group receiving the unsupplemented diet. Clinically, too, there was a marked improvement in the condition of the children in all groups during the course of the experiment, despite a low colorie intake; when discharged from the hospital they looked as fit and healthy as children of similar age and economic status. This is in marked contrast to the results obtained by Albanese and co-workers,[18] who also used a diet containing a high proportion of milk, and in agreement with the findings of Gaitonde et al.[38]

The ratio of lysine to threonine in the diets of these children varied from 1.4 to 1.5, which agrees with the value of 1.3 considered optimal for the rat.[39] During the period of lysine supplementation, however, this ratio ranged from 2.0 to 2.6 in groups 1 and 3, and yet the

children showed no effects of an amino acid imbalance;[40] the slight decrease in the rate of growth brought about by the addition of lysine in groups 1 and 3A (Table II) is not significant. Gaitonde et al.[38] obtained a more marked effect on adding lysine to the diet of patients with kwashiorkor, the average weight gain being 1.3 pounds during the three weeks of their experiment as compared with 3.2 pounds gained by the control group. Details of their "hospital diet" are not given.

That a mixture of amino acids rather than one amino acid is necessary to correct the deficiencies of a diet of highly polished rice has been established.[39,41,42] If such supplementation is to be effective in human nutrition the amino acids must be mixed with the whole grain and the grain then coated with a water-insoluble but digestible substance, as was done during the enrichment of rice with vitamins in the Phillipines,[43] to resist the effect of excessive washing of rice prior to cooking. Such a procedure will make amino acid supplementation very uneconomical. A more practical way of improving the value of a rice diet is to supplement it with foods containing well balanced proteins[42] or with proteins that can supply the deficiencies in the rice protein. Milk, although an excellent supplement, is scarce and expensive in underdeveloped countries, and deteriorates rapidly due to lack of effective refrigeration. Beef is cheaper but it is taboo to both Hindu and Buddhist. Of the various food supplements suggested[44] the following could be used in Ceylon: pulses, green leafy vegetables, fish and coconut.

Pulses

Several reports indicate the importance of pulses in the diets of pre-school and older children.[14,45–47] The most popular pulse in Ceylon is lentil (Lens esculenta); this is well tolerated by children provided it is well boiled. A relatively small amount can contribute substantially to the protein content of the diet. Unfortunately it is not grown in Ceylon, nearly all that is consumed being imported from India.

Leafy Vegetables

Although the protein content of green leaves is low, the protein is of a high biological value,[24] and green vegetables have been used successfully in supplementing rice diets.[48]

Fish

Fish is the one animal food that is acceptable to a large majority of the population. Sea fish is popular but the supply is insufficient, the prices high and the distribution largely confined to coastal districts. Only a few varieties of fresh water fish are eaten, and the supply is negligible. Fish, salted and dried, is commonly consumed in small quantities. The consumption of fresh water fish could be popularised, especially by breeding the more acceptable varieties in inland tanks and rivers and in rice fields during the period of inundation, as is done in Japan. Fish that is not popularly eaten when fresh could be either converted to fish meal or salted and dried.

Coconut

The residue left after extraction of the oil from the kernel has a high content of good quality protein, but its high fibre content makes it unsuitable for liberal use in the diets of young children. The production of a refined flour from coconut is at present engaging the attention of the Ceylon Institute for Scientific and Industrial Research, and it is hoped that the product will be suitable for addition to milk. It could be mixed with foods like manioc powder and fish meal. At present coconut is used in cooking in the form of "milk": the finely grated kernel is mixed with water and the "milk" expressed by hand, the process being repeated twice. Up to 80 per cent of the protein and most of the fat in the kernel are present in this milk[14] which is used for cooking vegetable and other curries. The feeding of more vegetables such as lentil and greens cooked in this milk would thus add to the protein in the diet. The milk has also been shown to be useful in the treatment of gastrointestinal disturbances in children.[49]

Increasing the local production of rice would make available more parboiled and lightly milled rice with a higher protein content than the highly milled white rice now imported. The germ and bran removed when polishing

rice is an effective supplement to white rice.[50] Good quality rice, if eaten in sufficient quantity, is apparently capable of keeping the population in positive health.[51]

Since World War II an increasing proportion of the population have taken to eating bread, at least one meal of bread per day being consumed in many homes. Wheat flour could be effectively enriched with several nutrients, including amino acids, provided a larger number of the poorer people could be persuaded to take bread at one of the meals, and the world's surplus of wheat was made available to them at a price within their means.

SUMMARY

Changes in body weight, thickness of subcutaneous tissue and in the concentration of proteins of the plasma have been investigated in five children recovering from kwashiorkor and in four from general undernutrition, prior to and during lysine supplementation of a milk plus rice and bread diet.

No difference in the rate of growth or in the improvement of plasma proteins was found between the two groups, or in the same group when results of the two experimental periods were compared. Nor was a difference noticed between these two groups and a third group of four undernourished children fed the same diet unsupplemented with lysine.

The intake of nutrients per day per kilogram body weight from the diet was as follows: calories 46 to 85, proteins 1.9 to 3.4 gm., lysine 115 to 200 mg. and threonine 77 to 142 mg.

The problem of improving the quality of rice diets fed to Ceylonese children is briefly discussed.

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