# Partition of Urinary Nitrogen in Children with Kwashiorkor Treated with Animal and Vegetable Proteins

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IN recent years a few investigations on the distribution of nitrogen in urine in patients with kwashiorkor have been reported.[1-3] These reports have stressed the importance of such studies elucidating the metabolic derangements in this disease. It is generally agreed that at the height of the disease there is a significant reduction in the urinary excretion of urea, and a relative increase in the amounts of other nitrogenous constituents. It was shown by Dean[2] that a large proportion of the nitrogen excreted in urine could not be accounted for by the common nitrogenous constituents, *viz.*, urea, ammonia, uric acid, creatinine, creatine and amino nitrogen; and it was claimed that the amount of undetermined nitrogen in urine was proportional to the severity of the disease and that after treatment little or no "undetermined nitrogen" remained. The output of abnormal quantities of purine bases was greatest in those patients in whom the amount of undetermined nitrogen was also the largest. On the basis of these observations Dean suggested that the unaccountable nitrogen in urine of patients with kwashiorkor may be attributable mainly to purine derivatives. On the other hand, Edozien and Phillips[3] tried to explain the substantial part of the undetermined nitrogen on the basis of the increased amounts of free and the bound forms of amino acids excreted in urine in kwashiorkor. These authors were able to account for practically all the undetermined nitrogen without allowing for purines; they attributed this to the nonspecificity of the uricase method which they employed for the estimation of uric acid.

These observations raise the fundamental question whether the increased excretion of nitrogenous constituents, such as ammonia, uric acid, purines and amino nitrogen, indicate an abnormality of nitrogen metabolism in kwashiorkor or whether they are physiologic reflections of a low level of nitrogen metabolism.

In the present report an attempt is made to elucidate information on major as well as minor nitrogen constituents of urine in patients with kwashiorkor before and after treatment with dietary proteins of vegetable and animal origin. Some of the natural metabolites of nitrogenous compounds like imidazole compounds and hippuric acid, which are excreted in normal human urine, have also been investigated in these studies.

## MATERIAL AND METHODS

### *Selection of Cases*

The patients with kwashiorkor selected for these studies were male children from one and a half to eight years of age; all exhibited the typical clinical characteristics of the disease of moderately severe degree. They were admitted to the metabolic ward and their diets were prepared in the kitchen attached to the unit. On admission the children were maintained on their home diets for twenty-four hours during which period the initial collections of urine were made. Dietary treatment was started after the first twenty-four–hour collection of urine

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was completed. Twenty-four-hour urine samples were also collected for analysis on the first day after the institution of treatment and on two consecutive days after clinical recovery at the completion of treatment (twenty-nine and thirty-four days). Whenever possible intermediate urine collections were made during the course of treatment which lasted for nearly four weeks in all the groups. Samples of blood were obtained for the determination of hemoglobin, total plasma protein and albumin both at the time of admission and at the time of the final urine collection.

### *Collection of Twenty-Four-Hour Urine*

An adaptor prepared from the thumb of a glove was used to ensure complete collection of twenty-four-hour urine samples. It was fixed over the perineum and pubic region of the child by means of adhesive cement. The penis and scrotum were kept within the stall and the tip of the tubular portion of the adaptor was attached through a glass connection to a polythene tube passing down to the collection bottle containing toluene. The bed was slightly inclined to facilitate the easy drainage of the urine into the bottle.

### *Analytical Methods*

Total nitrogen content of urine was estimated by the conventional macro-Kjeldahl procedure. Urea and ammonia content were determined by the aeration method of Van Slyke and Cullen.[4] Creatinine and creatinine plus creatine were estimated by the alkaline picrate method of Folin[5] before and after hydrolysis. Uric acid was determined by the colorimetric procedure of Benedict and Franke[6] and total amino acid nitrogen by the iodometric method of Albanese and Irby.[7] Hippuric acid in urine was extracted with ether in a liquid-liquid extractor and further estimated by Griffith's procedure.[8] Total imidazole compounds were determined by diazotization with sulphanilic acid using the colorimetric method described by Snell and Snell.[9] Total purine bases were precipitated as copper salts in alkaline medium and their nitrogen estimated[10] and a correction was applied for uric acid. Xanthine and guanine together were estimated by the micro-colorimetric method of Williams[11] and adenine by spectrophotometry.

### *Experimental Diets*

The patients were distributed at random into four groups and each group received a separate dietary treatment. It can be seen from Table I that the four diets used in these studies provided 48 to 53 gm. protein and 900 to 1,100 calories per day. Since the primary object of the investigation was to study the influence of different proteins of vegetable and animal origin on the nitrogen metabolism in kwashiorkor, peanut protein isolate formed the main source of protein in diet I, whereas in diets III and IV milk protein and milk and liver protein, respectively, predominated. In diet II, nearly 10 per cent of the peanut protein of diet I was substituted with milk protein.

TABLE I
Composition of Diets Used in the Treatment

| Components | Diet I (gm./day) | Diet II (gm./day) | Diet III (gm./day) | Diet IV (gm./day) |
|---|---|---|---|---|
| Peanut protein isolate* | 40 | 28 | ... | ... |
| Skim milk | ... | 14 | 112 | 40 |
| Liver protein concentrate† | ... | ... | ... | 28 |
| Bread | 168 | 168 | 168 | 168 |
| Brown sugar | 28 | 28 | 28 | 28 |
| Sago | 28 | 28 | 28 | 28 |
| Plantains | 50 | 50 | 50 | 50 |
| Proteins | | | | |
| Total | 48 | 53 | 53 | 52 |
| Vegetable | 48 | 48 | 14 | 14 |
| Animal | ... | 5 | 39 | 38 |
| Calories | 920 | 922 | 1,160 | 915 |

* Protein 86 per cent.
† Protein 85 per cent.

Skim milk was reconstituted to provide 3.5 gm. protein per 100 ml. and when peanut protein or liver protein concentrates were given along with milk, they were mixed with reconstituted milk and brown sugar and fed in three divided doses during the day. Peanut protein, when administered without milk (diet I), was mixed with water and brown sugar to form a gruel. A small quantity of trisodium phosphate was used to prevent sedimentation of particulate matter. All four diets proved to be highly acceptable to the children and had no disagreeable effects.

## RESULTS AND COMMENTS

### *Clinical and Biochemical Response of Patients to Treatment*

In patients receiving peanut protein isolate as a chief source of protein (group 1), the

TABLE II

Partition of Urinary Nitrogen in Patients with Kwashiorkor Treated with Peanut Protein Isolate (Group 1) and Peanut Protein Isolate with Skim Milk (Group 2)

| Nitrogen | Group 1 | | | Group 2 | | |
|---|---|---|---|---|---|---|
| | Before Treatment (mg.) | 1st Day of Treatment (mg.) | Last Day of Treatment (mg.) | Before Treatment (mg.) | 1st Day of Treatment (mg.) | Last Day of Treatment (mg.) |
| Total | 591.5 | 1,246.3 | 982.3 | 784.3 | 1,274.5 | 3,808.3 |
| Urea | 278.1 (47.0) | 539.4 (43.3) | 534.1 (54.3) | 420.2 (53.6) | 624.9 (49.0) | 2,514.1 (66.0) |
| Ammonia | 69.1 (11.7) | 66.4 (5.3) | 138.2 (14.1) | 93.1 (11.9) | 122.4 (9.6) | 166.1 (4.4) |
| Creatine | 11.0 (1.9) | 10.0 (0.8) | 17.6 (1.8) | 14.4 (1.8) | 50.1 (3.9) | 24.0 (0.6) |
| Creatinine | 9.3 (1.6) | 9.2 (0.7) | 14.8 (1.5) | 25.6 (3.3) | 32.8 (2.6) | 28.0 (0.7) |
| Amino | 51.5 (8.7) | 79.1 (6.3) | 66.7 (6.8) | 55.6 (7.1) | 84.5 (6.6) | 107.3 (2.8) |
| Hippuric acid | 3.5 (0.6) | 5.6 (0.5) | 8.0 (0.8) | 11.1 (1.4) | 11.4 (0.9) | 17.6 (0.5) |
| Total imidazole (in terms of histidine) | 14.3 (2.4) | 17.6 (1.4) | 15.7 (1.6) | 17.9 (2.3) | 20.0 (1.6) | 15.5 (0.4) |
| Purine (without uric acid) | 2.4 (0.4) | 42.9 (3.4) | 1.1 (0.1) | 26.1 (3.3) | 45.5 (3.6) | 37.0 (1.0) |
| Uric acid | 18.0 (3.0) | 13.8 (1.1) | 46.1 (4.7) | 32.2 (4.1) | 46.9 (3.7) | 73.0 (1.9) |
| Xan-guanine | 0.7 | 1.7 | 1.6 | 1.6 | 5.0 | 7.9 |
| Adenine | 2.0 | 2.1 | 1.5 | 3.4 | 2.4 | 7.4 |
| Undetermined | 134.3 (22.7) | 458.9 (36.8) | 140.5 (14.3) | 88.1 (11.2) | 266.2 (20.9) | 825.8 (21.7) |

NOTE: Values given are based on the average of two patients. Figures in parentheses indicate percentage of total nitrogen.

edema persisted beyond twelve days and there was no evidence of regeneration of plasma proteins. In contrast, those who received the other three diets, which contained the mixture of peanut protein and milk, milk alone or milk plus liver protein, showed a relatively good response. Edema disappeared within five to ten days and there was a consistent increase in hemoglobin, total protein and albumin of plasma. Gain in body weight after disappearance of edema was observed in these three groups, particularly in those who received the milk and liver protein. The children in the latter group were apparently more active and alert. It was observed that the response to treatment with peanut protein was not very successful unless some source of animal protein was also included in the mixture.

### *Partition of Urinary Nitrogen Before and After Treatment*

The average values for the different nitrogenous constituents in urine of children treated with the four diets are given in Tables II and III. The output of total urinary nitrogen in the nine patients studied ranged from 415 to 839 mg. for twenty-four hours before the treatment; these low values were in keeping with the low nitrogen intake by these patients on their home diets. These results are in general agreement with those of Platt[1] and Dean.[2] However, no difference was observed in the nitrogen excretion of those who had "severe" and those who had "less severe" cases as reported by Dean.[2] It must be pointed out that the criteria for the assessment of "severity" in kwashiorkor are debatable.

The excretion of total nitrogen nearly doubled in the first twenty-four hours after treatment was started. The children who received the vegetable protein excreted a larger proportion of the ingested nitrogen in urine than those who received the animal protein diets. The poor retention of vegetable protein in the early stages of treatment was evident from these results. There was a marked increase in the urinary excretion of nitrogen after treatment, particularly in the children treated with animal proteins and

TABLE III

Partition of Urinary Nitrogen in Patients with Kwashiorkor Treated with Skim Milk (Group 3) and Skim Milk with Liver Protein (Group 4)

| Nitrogen | Group 3 | | | Group 4 | | |
|---|---|---|---|---|---|---|
| | Before Treatment (mg.) | 1st Day of Treatment (mg.) | Last Day of Treatment (mg.) | Before Treatment (mg.) | 1st Day of Treatment (mg.) | Last Day of Treatment (mg.) |
| Total | 429.0 | 691.8 | 4,275.3 | 708.2 | 905.3 | 2,449.4 |
| Urea | 231.9 (54.1) | 277.8 (40.2) | 3,518.8 (82.3) | 307.7 (43.4) | 457.3 (50.5) | 1,986.4 (81.1) |
| Ammonia | 67.8 (15.8) | 108.7 (15.7) | 249.9 (5.8) | 122.8 (17.3) | 113.3 (12.5) | 161.0 (6.6) |
| Creatine | 1.6 (0.4) | 73.0 (10.6) | 56.6 (1.3) | 10.9 (1.5) | 17.9 (2.0) | 32.4 (1.3) |
| Creatinine | 12.5 (2.9) | 26.6 (3.8) | 23.1 (0.5) | 29.0 (4.1) | 19.9 (2.2) | 72.3 (3.0) |
| Amino | 40.6 (9.5) | 70.8 (10.2) | 61.9 (1.4) | 66.2 (9.3) | 47.1 (5.2) | 95.3 (3.9) |
| Hippuric acid | 1.9 (0.4) | 3.0 (0.4) | 15.6 (0.4) | 7.0 (1.0) | 7.1 (0.8) | 9.8 (0.4) |
| Total imidazole (in terms of histidine) | 10.5 (2.4) | 13.4 (1.9) | 24.1 (0.6) | 12.4 (1.8) | 6.9 (0.8) | 13.8 (0.6) |
| Purine (without uric acid) | 22.6 (5.3) | 26.9 (3.9) | 66.7 (1.6) | 37.0 (5.2) | 18.3 (2.0) | 64.4 (2.6) |
| Uric acid | 12.6 (2.9) | 25.0 (3.6) | 20.2 (0.5) | 19.2 (2.7) | 19.1 (2.1) | 26.3 (1.1) |
| Xan-guanine | 0.1 | 0.2 | 0.4 | 0.6 | 0.5 | 2.3 |
| Adenine | 2.1 | 3.5 | 4.7 | 1.4 | 1.0 | 5.8 |
| Undetermined | 27.0 (6.4) | 122.2 (17.7) | 238.3 (5.6) | 96.0 (13.6) | 197.9 (21.9) | Nil |

NOTE: Values given in group 3 are based on the average of three patients and in group 4 of two patients. Figures in parentheses indicate percentage of total nitrogen.

with the animal protein and peanut protein combination. The smallest increase was observed in those who received peanut protein alone.

### *Urea Nitrogen*

It can be seen from the results that the twenty-four–hour urea nitrogen level at the time of admission ranged between 43 and 54 per cent of the total urinary nitrogen. In six of nine patients the amount of nitrogen appearing as urea was less than 50 per cent. Although during the first twenty-four hours of treatment the total nitrogen rose appreciably, in most patients the percentage of urea nitrogen did not show any difference and the relative concentration of the other constituents of the urine also remained unchanged on the first day of treatment. After clinical recovery, however, the urea nitrogen showed considerable increase both in absolute quantities as well as percentage of total nitrogen. The rise was marked particularly in children receiving milk, and milk and liver protein diets and was only slight in those receiving peanut protein. Therefore, it may be permissible to conclude that apart from the difference in the speed of plasma albumin regeneration reported earlier,[12] the difference in the increase of the proportion of urea nitrogen to total urinary nitrogen after treatment was another demonstrable biochemical difference between the response of patients with kwashiorkor to vegetable and animal protein diets.

There appeared to be a correlation between the percentage of urea nitrogen in twenty-four–hour urine samples and the plasma albumin. When the data for all observations before and after treatment were pooled (sixteen in all), a significant correlation was established between the plasma albumin value and urea nitrogen ($\gamma = 0.694$) at the 1 per cent level. Admittedly, the deviations are considerable and a larger number of observations would have brought out a better correlation between plasma albumin and urinary urea. The regression equation is given in Figure 1, and the actual observations have been plotted to illustrate this relationship. If depression

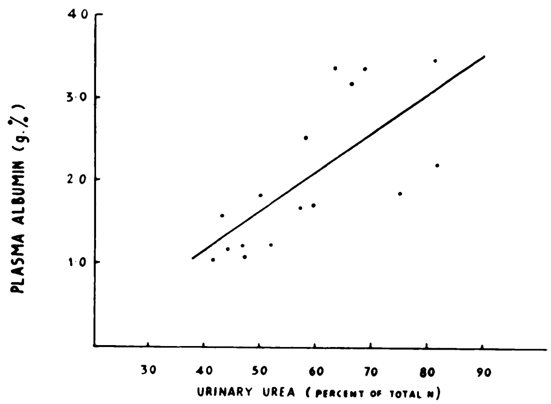


FIG. 1. Relationship between plasma albumin and percentage of urea nitrogen in twenty-four hours. Urine of patients with kwashiorkor. Correlation coefficient = + 0.694; regression equation Y = 0.0475 X − 0.7225.

in serum albumin level can be considered a criterion of the severity of protein malnutrition it may be argued that the proportion of urea nitrogen to total nitrogen also may be considered as an index of the severity of protein depletion.

### *Ammonia Nitrogen*

The ammonia nitrogen ranged from 68 to 123 mg. in twenty-four hours, constituting 12 to 17 per cent of total urinary nitrogen before treatment was started. After treatment with the animal protein diet, the percentage excretion of ammonia nitrogen returned to the normal level of about 5 to 6 per cent. These results agree with the findings of other workers. On the peanut protein diet, where the response to treatment was poor, the level of ammonia nitrogen in urine remained high.

### *Amino Nitrogen*

In six of nine patients, aminoaciduria of varying degree was observed and amino nitrogen excretion ranged from 8 to 12 per cent of the total urinary nitrogen. After treatment, although the absolute figures for amino nitrogen showed a slight increase, their relative proportion to total nitrogen showed a marked reduction. In this respect our results agree with those of Edozien and Phillips.[3]

### *Other Nitrogenous Constituents*

In the present study creatine, creatinine, hippuric acid, total imidazole compounds, uric acid and purines were excreted in relatively increased quantities by all the patients with kwashiorkor investigated. These constituents together formed an appreciable proportion of the total urinary nitrogen which might be a reflection of catabolism of body proteins in the absence of adequate dietary protein. Whitehead and Arnstein[13] observed increased excretion of imidazole acrylic acid in the urine of children with kwashiorkor and it is postulated that the histidine metabolism is disturbed in severely ill children. In the present studies, total imidazole compounds were estimated using histidine as reference standard. The procedure of diazotization with sulphanilic acid adopted in the determination of imidazole compounds would have estimated imidazole acrylic acid. These studies did not reveal any abnormality in histidine metabolism in kwashiorkor. Further work, however, is necessary using more specific methods before any definite conclusions are drawn.

Excretion of creatine, creatinine, hippuric acid and imidazole compounds remained remarkably constant during the period of treatment. On the other hand, an appreciable rise in the excretion of uric acid and purine derivatives was observed for a long period during treatment. However, the percentage excretion of uric acid and purines fell considerably during the treatment period, particularly when animal protein was present in the diet. The inclusion of nucleoprotein in the diet of the children in group 4 did not result in a greater excretion of purines after treatment as compared to the children who were treated with milk or milk and peanut protein. These results would indicate that the purines of exogenous origin were normally metabolized by malnourished children. The initial high level of purine bases in only some cases would show that purine metabolism might be affected at some stage in kwashiorkor. The excretion of individual purine bases, *viz.*, xanthine, guanine and adenine, was only slightly altered in these patients which suggested that probably the methylated purine derivatives could be the major purine compounds. Our observations, however, do not substantiate the views of Dean[2] that in children suffering from kwashiorkor the excretion of certain abnormal purine derivatives is considerably increased, the amount excreted bearing a direct relationship with the severity of the disease. In our series we could detect large amounts of purine derivatives in the urine of only one patient. In three of nine patients purine excretion was below normal levels.

### *Undetermined Nitrogen*

It was generally observed that the patients with kwashiorkor excreted large amounts of nitrogen in urine which could not be accounted for by the constituents estimated. Here again the results were not uniform in all cases. In four patients undetermined nitrogen was below 5 per cent, and in the remaining five it represented from 11 to 20 per cent of total urinary nitrogen. The severity of the disease did not apparently influence the level of undetermined nitrogen in the urine. It was interesting to observe that the undetermined nitrogen remained at a high level after treatment with predominantly vegetable protein diet (14 and 21 per cent in groups 1 and 2). On the other hand, with animal protein diets only a small proportion, nearly 3 to 8 per cent of total nitrogen, was eliminated in urine which could not be accounted for by the nine constituents estimated in these studies.

It can be inferred from the present investigation that kwashiorkor is in general associated with low values of urea nitrogen/total urinary nitrogen. The increase in the relative amounts of ammonia nitrogen, free amino acid nitrogen and undetermined nitrogen in urine also could be considered as common features of kwashiorkor. These abnormal changes in the nitrogenous constituents could be a reflection of prolonged low protein intake and a low level of nitrogen metabolism. The results of urinary nitrogen partition for the first few days of treatment suggested that the protein deficient organs were incapable of handling the dietary protein efficiently.

In the present studies we have attempted to estimate some more minor nitrogenous constituents, *viz.*, hippuric acid and imidazole compounds in addition to purine derivatives and free amino acids. However, the amount of undetermined nitrogen which was high in most patients before treatment was not completely eliminated after treatment with good quality protein. Neither purine derivatives nor amino acid nitrogen could account for the initial high level of undetermined nitrogen. The investigation further brought to light the fact that the response of patients with kwashiorkor to treatment with different proteins could be indicated by the pattern of urinary nitrogen. In this respect, the direct correlation observed between plasma albumin values and percentage of urea nitrogen in twenty-four–hour urine samples is significant.

## SUMMARY

The pattern of urinary nitrogen partition in patients with kwashiorkor is studied. The effect of different lines of treatment on this pattern are investigated.

Nine patients (distributed into four groups) were treated with one of four diets, the protein moiety consisting of either peanut protein isolate, peanut protein plus skim milk, skim milk alone or skim milk plus liver protein. Twenty-four–hour collections of urine were analyzed before commencement of treatment and periodically during treatment for nine nitrogenous constituents, including certain minor compounds, *viz.*, purine derivatives, imidazole compounds and hippuric acid.

Nitrogen partition at the height of the disease showed a low percentage of urea nitrogen and a relatively high percentage of ammonia nitrogen, amino nitrogen and undetermined nitrogen.

Total urinary nitrogen increased appreciably even on the first day of treatment but the pattern of nitrogen partition remained unaffected.

After clinical recovery, there was a considerable rise in urea nitrogen. It was particularly marked in patients receiving animal protein diets and only slight in those receiving vegetable protein diets.

Ammonia nitrogen and amino nitrogen returned to normal levels only after successful treatment with animal proteins and animal and vegetable protein mixtures. The response to treatment with peanut protein isolate in this respect was poor.

Treatment did not result in the elimination of undetermined nitrogen although it brought about its reduction when animal protein diets were employed.

A direct correlation was observed between percentage of urea nitrogen in urine and plasma albumin values before and after treatment. This would suggest that the percentage of urea nitrogen may be as good an index of the severity of kwashiorkor as the serum albumin level.

ACKNOWLEDGMENT

We are grateful to Dr. C. Gopalan, Director, Nutrition Research Laboratories, Hyderabad, India, for his keen interest in the work, and to Dr. S. G. Srikantia for his help in the collection of the material for the studies.

We wish to thank Biological Products Ltd., Hyderabad, India, for the supply of liver protein concentrate used in these studies. Thanks are due to the Director, Central Food Technological Research Institute, Mysore, India, for the supply of peanut protein isolate.

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