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# SUPPLEMENTATION OF CEREAL PROTEINS WITH AMINO ACIDS

## II. EFFECT OF AMINO ACID SUPPLEMENTATION OF CORN-MASA AT INTERMEDIATE LEVELS OF PROTEIN INTAKE ON THE NITROGEN RETENTION OF YOUNG CHILDREN [1]

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The first paper in this series (Scrimshaw et al., '58) presented the results of supplementing corn protein fed to young children at a level of 3.0 gm per kilogram of body weight with synthetic essential amino acids to match the amino acid pattern of the F.A.O. "reference protein" ('57). Although nitrogen retention was poor on the basal diet alone, it was progressively improved by the step-wise addition of tryptophan, lysine, and isoleucine. On one case the basal diet supplemented with tryptophan, lysine and isoleucine was compared with one in which the same amount of protein was furnished by milk; greater nitrogen retention was found with the former than with the milk-based diet. In general, the children tended to gain weight on the corn-masa diet when the amino acid retention became or remained positive as the result of the amino acid supplementation.

Recent reports suggest that to some extent, merely increasing the intake of a poor quality protein can compensate for part of its qualitative deficiency and result in greater absolute

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nitrogen retention (Allison and Anderson, '45; De Maeyer and Vanderborght, '57; Sure, '57). At the same time, with high levels of intake, the relative percentage of retention was improved and the distinction between the nitrogen retention of proteins of widely differing biological value was shown to be less marked. In other words, when fed at high intake levels, differences in the amino acid composition of the diet appear to become less critical and less easy to determine.

This second paper explores the effect of amino acid supplementation at levels of 2.0 and 1.5 gm of protein per kilogram which are relatively low for children of this age and physiological state (Béhar, Viteri and Scrimshaw, '57). In general the results confirm those obtained previously at the level of 3.0 gm of protein per kilogram of body weight, but the responses to the amino acid additions are of smaller magnitude.

## MATERIAL AND METHODS

The techniques and diet employed were identical with those described previously (Scrimshaw et al. '58) except that less protein was supplied, and two successive three-day collection periods were employed with each combination tested. Two comparison periods in which the only protein in the diet came from milk were available as part of other experimental work. In these two cases 5-day balance periods were begun at the end of 7 days on the milk diet so that the results should represent stabilized values. Case PC-66, a boy one year and 10 months old weighing 8.9 kg and measuring 71.5 cm at the beginning of the trials and child PC-67, one year and 6 months of age weighing 9.2 kg and measuring 72.0 cm were both fed 2.0 gm of protein and 90 Cal. per kilogram per day. PC-57, a 4-year and 9-months old boy weighing 15.6 kg with a height of 89.5 cm, when the series of trials was begun, was fed a diet providing 1.5 gm of protein and 80 Cal. per kilogram of body weight per day.

## RESULTS

*Case PC-67.* The responses of child PC-67 are shown in figure 1 in which the data for each pair of three-day periods are averaged. The initial milk balance based on a 5-day period showed a nitrogen retention of 36 mg per kilogram per day or 8.2% of the amount consumed, compared with —15 mg per kilogram per day and —5.7% during the 6 days on the

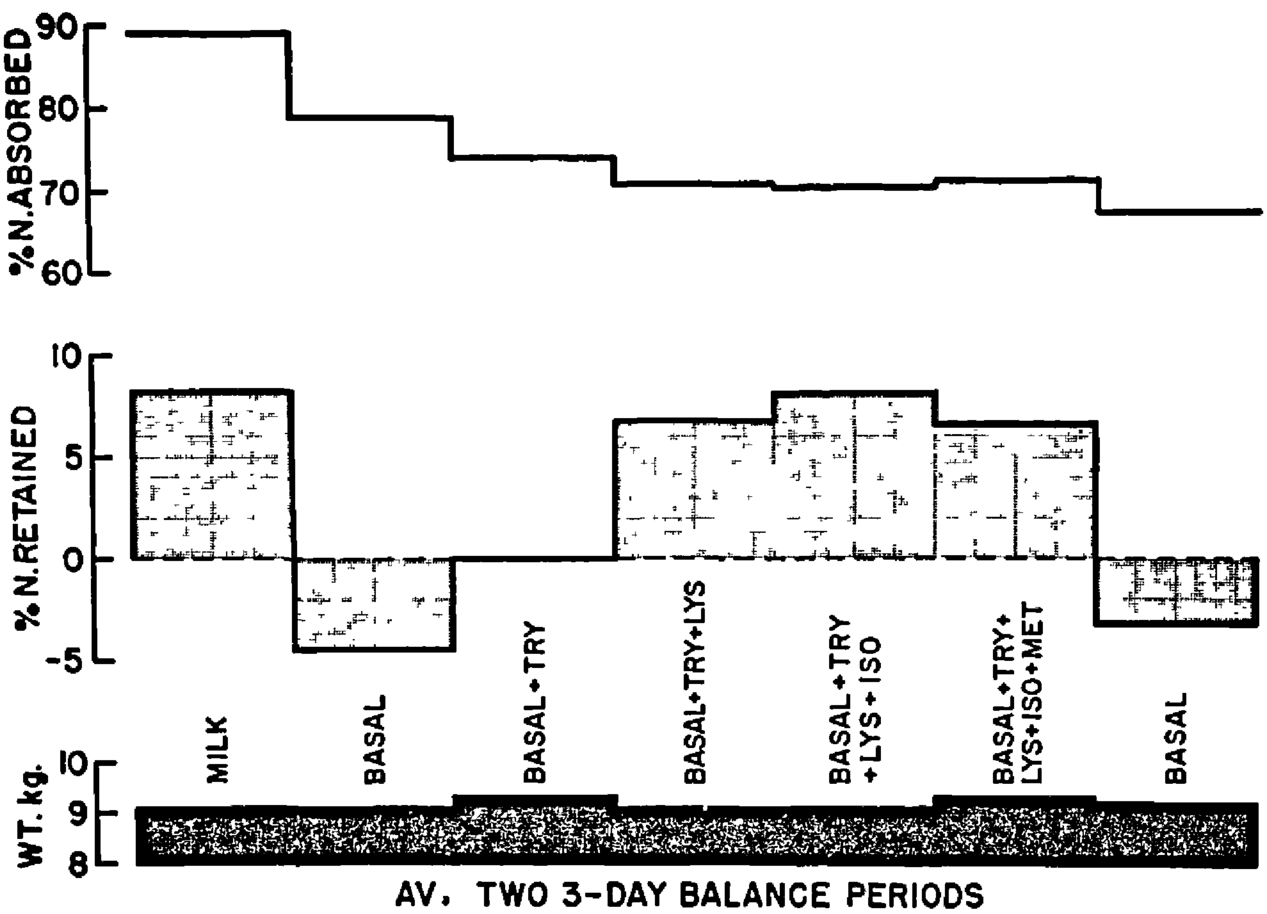


Fig. 1 Nitrogen absorption and retention and weight changes in child PC-67 recently recovered from kwashiorkor and fed milk or a corn-masa basal diet progressively supplemented with amino acids.

basal diet. When tryptophan was added in an amount designed to bring the total of this amino acid in the diet to that of the F.A.O. "reference protein" ('57), nitrogen retention improved to the point where the child was in nitrogen balance. The addition of 0.56% of lysine to the tryptophan supplement resulted in a marked further improvement in nitrogen retention; adding isoleucine brought about a slight additional increase to a level of retention nearly comparable to that found with milk protein at the beginning of the trials. Adding

TABLE 1

*Daily intake, excretion and retention of nitrogen by PC-67 with three-day periods of supplementation*

| THREE-DAY PERIOD | DIET | NITROGEN INTAKE | NITROGEN IN FECES | NITROGEN ABSORBED | NITROGEN ABSORBED | NITROGEN IN URINE | NITROGEN RETAINED | NITROGEN RETAINED | WEIGHT |
|---|---|---|---|---|---|---|---|---|---|
| | | *mg/kg/day* | | | *% intake* | *mg/kg/day* | | *% intake* | *kg* |
| | Milk [1] | 438 | 47 | 391 | 89.3 | 355 | + 36 | + 8.2 | 9.10 |
| 1 | Basal | 319 | 61 | 258 | 80.9 | 260 | — 2 | — 0.6 | 9.10 |
| 2 | Basal | 319 | 70 | 249 | 78.2 | 277 | — 28 | — 8.6 | 9.10 |
| 3 | Basal + tryptophan | 319 | 73 | 246 | 77.1 | 235 | + 11 | + 3.4 | 9.29 |
| 4 | Basal + tryptophan | 319 | 93 | 226 | 70.8 | 237 | — 11 | — 3.4 | 9.29 |
| 5 | Basal + tryptophan + lysine | 322 | 86 | 236 | 73.3 | 196 | + 40 | + 12.4 | 9.12 |
| 6 | Basal + tryptophan + lysine | 322 | 102 | 220 | 68.3 | 215 | + 5 | + 1.6 | 9.12 |
| 7 | Basal + tryptophan + lysine + isoleucine | 322 | 99 | 223 | 69.2 | 199 | + 24 | + 7.4 | 9.12 |
| 8 | Basal + tryptophan + lysine + isoleucine | 322 | 91 | 231 | 71.7 | 204 | + 27 | + 8.4 | 9.12 |
| 9 | Basal + tryptophan + lysine + isoleucine + methionine | 317 | 94 | 223 | 70.4 | 197 | + 26 | + 8.2 | 9.26 |
| 10 | Basal + tryptophan + lysine + isoleucine + methionine | 317 | 87 | 230 | 72.6 | 212 | + 18 | + 5.7 | 9.26 |
| 11 | Basal | 319 | 98 | 221 | 69.3 | 220 | + 1 | + 0.3 | 9.20 |
| 12 | Basal | 319 | 112 | 207 | 64.9 | 228 | — 21 | — 6.6 | 9.20 |

[1] Five-day balance period

methionine to the mixture of supplementary amino acids brought about a small drop which is apparent in figure 1, and returning the child to the basal diet resulted once again in negative nitrogen balance. The results are more variable when the data are examined for the two separate three-day periods averaged for figure 1. The data for each three-day

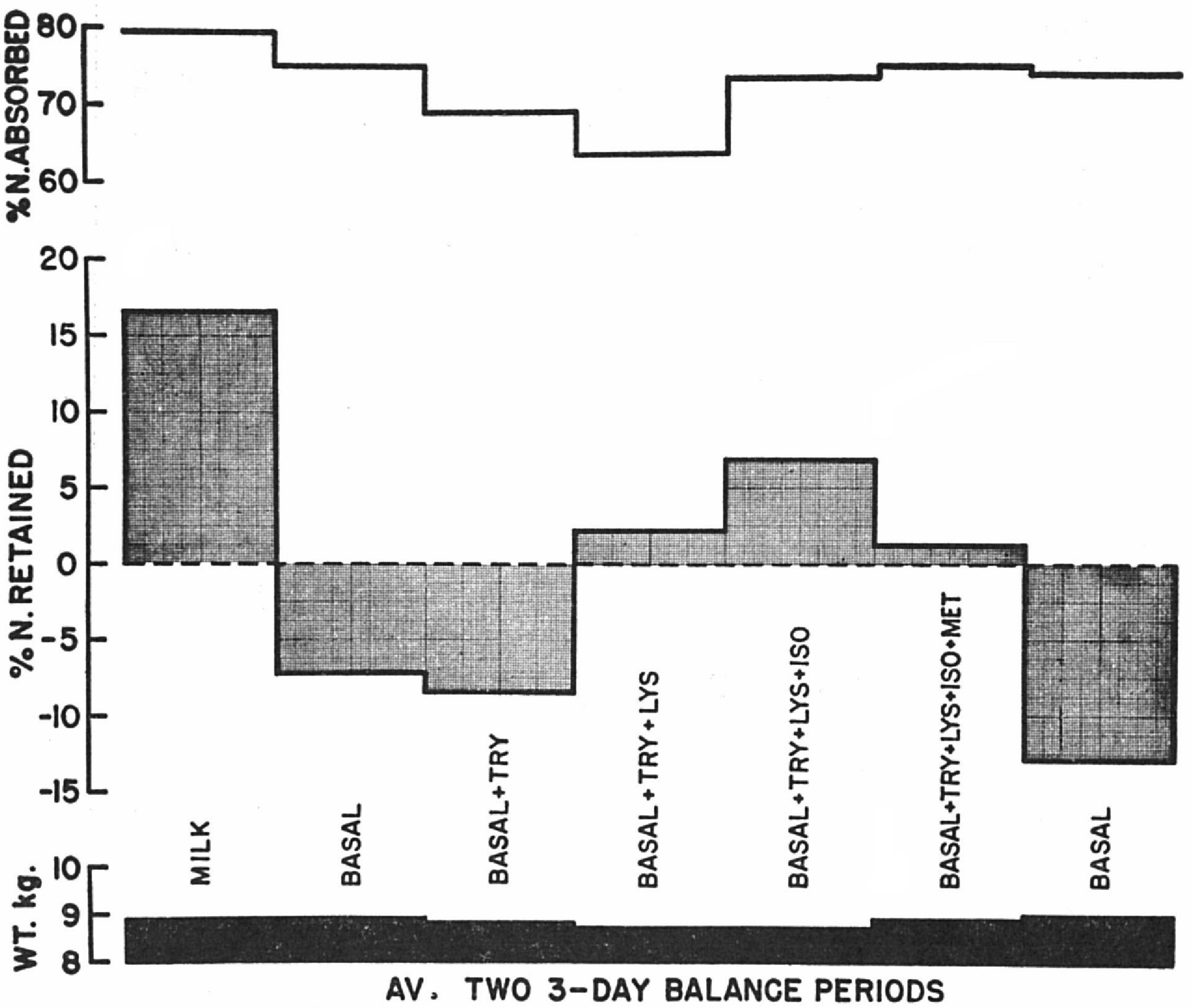


Fig. 2 Nitrogen absorption and retention and weight change in child PC-66 recently recovered from kwashiorkor and fed milk or a corn-masa basal diet progressively supplemented with amino acids.

period as tabulated in table 1 suggest a tendency for the responses to be greater in the first three-day period on an experimental treatment than during the second, although the results are not entirely consistent. Absorption of nitrogen varied from 65 to 81% without a clear pattern emerging.

*Case PC-66.* The response of PC-66 to progressive supplementation of corn-massa protein with synthetic amino acids is shown in figure 2. The changes proved similar to

TABLE 2

*Daily intake, excretion and retention of nitrogen by PC-66 with three-day periods of supplementation*

| THREE-DAY PERIOD | DIET | NITROGEN INTAKE | NITROGEN IN FECES | NITROGEN ABSORBED | NITROGEN ABSORBED | NITROGEN IN URINE | NITROGEN RETAINED | NITROGEN RETAINED | WEIGHT |
|---|---|---|---|---|---|---|---|---|---|
| | | *mg/kg/day* | | | *% intake* | *mg/kg/day* | | *% intake* | *kg* |
| | Milk [1] | 432 | 90 | 342 | 79 2 | 270 | + 72 | + 16.7 | 8 90 |
| 1 | Basal | 321 | 82 | 239 | 74 3 | 265 | — 26 | — 8 3 | 8.77 |
| 2 | Basal | 321 | 61 | 260 | 81 0 | 278 | — 18 | — 5.6 | 8 77 |
| 3 | Basal + tryptophan | 321 | 101 | 220 | 68 5 | 241 | — 21 | — 6 5 | 8 75 |
| 4 | Basal + tryptophan | 321 | 97 | 224 | 69 7 | 253 | — 29 | — 9 0 | 8 75 |
| 5 | Basal + tryptophan + lysine | 320 | 118 | 202 | 63 2 | 199 | + 3 | + 0 9 | 8 69 |
| 6 | Basal + tryptophan + lysine | 320 | 114 | 206 | 64 5 | 194 | + 12 | + 3 8 | 8 69 |
| 7 | Basal + tryptophan + lysine + isoleucine | 320 | 89 | 231 | 72 3 | 209 | + 22 | + 6 8 | 8 69 |
| 8 | Basal + tryptophan + lysine + isoleucine | 320 | 81 | 239 | 74 6 | 216 | + 23 | + 7 2 | 8 69 |
| 9 | Basal + tryptophan + lysine + isoleucine + methionine | 312 | 87 | 225 | 72 1 | 235 | — 10 | — 3 2 | 8 92 |
| 10 | Basal + tryptophan + lysine + isoleucine + methionine | 312 | 70 | 242 | 77 5 | 223 | + 19 | + 6 1 | 8 92 |
| 11 | Basal | 320 | 83 | 237 | 74 1 | 278 | — 41 | — 12 9 | 9 00 |

[1] Five-day balance period

those in figure 1, except that there was no significant response to the initial addition of tryptophan. The supporting data tabulated by three-day periods are shown in table 2. There was the same tendency as in the preceding series for the first three-day period of each pair to show a more marked response to the changes in the diet than the second. With the exception

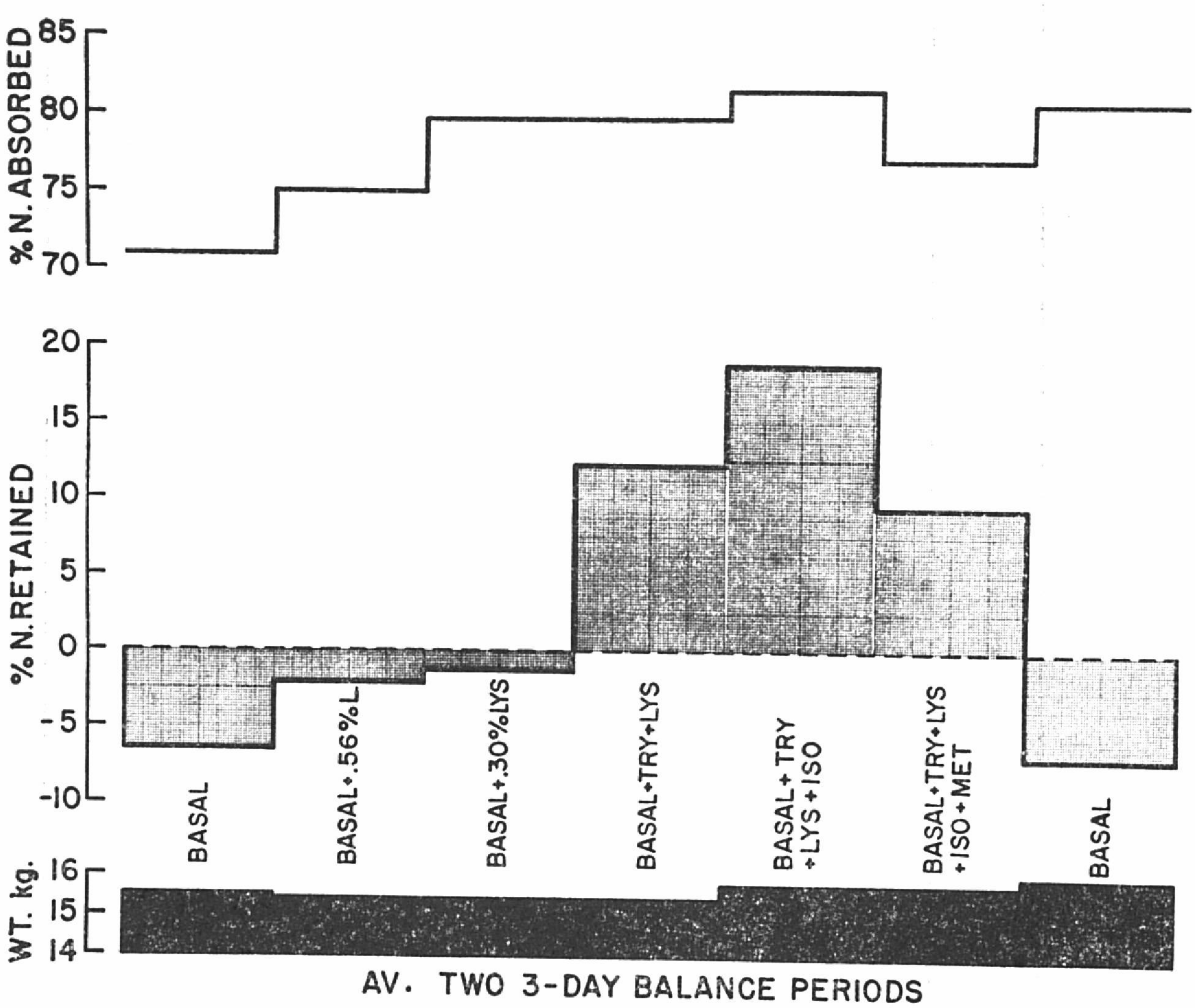


Fig. 3 Nitrogen absorption and retention and weight changes in child PC-57 recently recovered from kwashiorkor and fed a corn-masa basal diet progressively supplemented with amino acids.

of the initial supplementation with tryptophan, essentially no difference in nitrogen retention appeared between each of the two three-day periods when using combinations of tryptophan, lysine and isoleucine. The amount and percentage of nitrogen retained was greater with milk than with any pair of experimental periods involving the basal diet in this series of trials. Nitrogen absorption was 79% during the initial milk period and ranged from 63 to 81% thereafter.

TABLE 3

*Daily intake, excretion and retention of nitrogen by PC-57 with three-day periods of supplementation*

| THREE-DAY PERIOD | DIET | NITROGEN INTAKE | NITROGEN IN FECES | NITROGEN ABSORBED | NITROGEN ABSORBED | NITROGEN IN URINE | NITROGEN RETAINED | NITROGEN RETAINED | WEIGHT |
|---|---|---|---|---|---|---|---|---|---|
| | | | *mg/kg/day* | | *% intake* | *mg/kg/day* | | *% intake* | *kg* |
| 1 | Basal | 241 | 73 | 168 | 69.9 | 190 | — 22 | — 8.8 | 15.6 |
| 2 | Basal | 241 | 69 | 172 | 71.6 | 185 | — 13 | — 5.2 | 15.6 |
| 3 | Basal + 0.56% lysine | 239 | 68 | 171 | 71.5 | 183 | — 12 | — 5.0 | 15.5 |
| 4 | Basal + 0.56% lysine | 239 | 51 | 188 | 78.5 | 185 | + 3 | + 1.3 | 15.5 |
| 5 | Basal + 0.30% lysine | 239 | 52 | 187 | 78.4 | 196 | — 9 | — 3.5 | 15.5 |
| 6 | Basal + 0.30% lysine | 239 | 45 | 194 | 81.0 | 191 | + 3 | + 1.2 | 15.5 |
| 7 | Basal + tryptophan + lysine | 239 | 49 | 190 | 79.5 | 151 | + 39 | + 16.3 | 15.5 |
| 8 | Basal + tryptophan + lysine | 239 | 46 | 193 | 80.8 | 173 | + 20 | + 8.4 | 15.5 |
| 9 | Basal + tryptophan + lysine + isoleucine | 240 | 45 | 195 | 81.2 | 141 | + 54 | + 22.5 | 15.8 |
| 10 | Basal + tryptophan + lysine + isoleucine | 240 | 43 | 197 | 82.2 | 160 | + 37 | + 15.3 | 15.8 |
| 11 | Basal + tryptophan + lysine + isoleucine + methionine | 240 | 55 | 185 | 76.9 | 152 | + 33 | + 13.6 | 15.8 |
| 12 | Basal + tryptophan + lysine + isoleucine + methionine | 240 | 55 | 185 | 76.9 | 172 | + 13 | + 5.2 | 15.8 |
| 13 | Basal | 235 | 49 | 186 | 79.1 | 188 | — 2 | — 0.9 | 16.1 |
| 14 | Basal | 235 | 42 | 193 | 82.1 | 199 | — 6 | — 2.6 | 16.1 |

*Case PC-57.* This child differed from the others by being older and heavier. Thus the child's protein intake level of 1.5 gm per kilogram probably has a similar relation to requirement as did the 2.0 gm per kilogram for the two younger children. The results as shown in figure 3 are similar in general findings to those found in the previous trials. In this case, however, the diet was supplemented first with lysine instead of tryptophan. Adding sufficient lysine to bring the total of this amino acid up to the amount present in the "reference protein" pattern had a small positive effect on nitrogen retention, and reducing the lysine supplementation by roughly half did not result in any significant change. The addition of tryptophan to the basal diet plus lysine, however, produced a much greater response and the addition of isoleucine increased the net nitrogen retention to a level comparable to that which would have been expected with milk.

The addition of methionine again produced a distinct drop in nitrogen retention and retention became strongly negative when the child was returned to the basal diet. The data for individual three-day periods shown in table 3 reveal some variation between pairs but not enough to alter the conclusions drawn from the average of both. Nitrogen absorption varied from 70 to 82% in this series of trials.

## DISCUSSION

The nitrogen retention data presented show that the responses of children fed corn-masa protein supplemented with various synthetic amino acid combinations are qualitatively the same at intake levels of 2.0 and 1.5 gm as those previously reported (Scrimshaw et al., '58) for experiments at 3.0 gm of protein per kilogram per day. However, at the intermediate levels of protein intake employed in the present study, the basal diet resulted in strongly negative balances in each case. As reported previously for higher protein intakes step-wise supplementation of the basal diet with tryptohan, lysine and isoleucine to match the amino acid pattern of the F.A.O.

"reference protein" ('57) resulted in step-wise increases in the average nitrogen retention for two three-day periods. In general, the second three-day period gave a higher retention than the first when the change resulted in a net improvement. Conversely, the second three-day period gave a lower retention when the change resulted in a net decrease as with the addition of methionine or the return to the basal diet alone.

It is clear from the data that single three-day periods are sufficient to indicate the direction of the response. However, these tendencies are emphasized by the use of two three-day periods. For most purposes it would be sufficient to determine nitrogen balance for three days after a 5-day adjustment period; the results of amino acid supplementation would be qualitatively the same as with the use of a two-day adjustment period but would present even greater quantitative contrasts. Therefore, the effects of amino acid supplementation on nitrogen retention are more consistent when the results of the second three-day balance period are examined. This observation was also noted by Barness et al. ('57) who state that less variable retentions are obtained in the second three-day interval of their two three-day period studies with children. From a physiological point of view, a better picture of the mechanism of protein absorption and utilization is obtained when information from the two periods is used separately.

Although the weight gains were not as consistent as previously reported from the higher level of protein intake, it is important to note that these children, consuming corn-masa protein as a sole protein source at a rate of 1.5 to 2.0 gm of protein per kilogram, showed no significant loss of weight. It is of considerable interest that the children lost more nitrogen on the basal diet at the levels used in this study than when 3.0 gm per kilogram per day were employed as in the first paper in this series. Similarly the addition of the amino acids tryptophan or lysine or both, did not have as great an effect on the actual amount of nitrogen retained as that reported previously for the higher levels of protein intake. It is possible that adding a single amino acid to the level of the F.A.O. pattern ('57), without at the same time correcting deficiencies of other amino acids, could create a new imbalance; under such circumstance nitrogen retention could be stationary or even decreased. An imbalance associated with the addition of a single amino acid would be most apparent in diets low in nitrogen (Harper, '58). This may be an explanation for the poorer response to supplementation of the basal diet with tryptophan alone at the lower nitrogen intake of the present study.

The combination of tryptophan, lysine and isoleucine produced excellent results in two of the cases, PC-57 and PC-67. In the trials with PC-66, however, unlike the other experiments to date, this combination of amino acids did not bring the nitrogen retention near the range previously obtained with milk. We cannot at present offer any explanation for the consistently lower retention figures of this child with the basal diet and its supplemented combinations unless it reflects genetic variation, incomplete recovery, or some lingering damage from the severe disease which he has experienced. It may also be significant that the protein intake of this child was believed to be lower in proportion to his body needs than in any of the other trials.

In interpreting any of the results using children of varying ages recovering from malnutrition, it is important to note that a given level of protein intake may be high for one child, intermediate for another and low for a third depending on weight and state of nitrogen stores. It must also be noted that when these children begin the study they may still be relatively depleted in protein so that the percentage of nitrogen retained on a given intake slowly drops as nitrogen stores are replenished. This had been taken into account in both the experimental design and the interpretation of the data. The rate of this change varies greatly from child to child and disappears after prolonged treatment. As experience accumulates, it will perhaps be possible to analyze and generalize more on the quantitative significance of these changes resulting from amino acid supplementation. From a qualitative stand point, meanwhile, the results show that the supplementation of a cereal protein of relatively poor biological value such as corn with appropriate amino acids results in a consistent improvement in the nitrogen retention of young children fed the intermediate levels for their age and physiological state of 1.5 to 2.0 gm of protein, and 80 to 90 Cal. respectively per kilogram of body weight.

## SUMMARY

Two boys aged 22 and 18 months, weighing 8.9 and 9.2 kg, respectively and recently recovered from severe protein malnutrition (kwashiorkor), were fed a simplified basal diet providing 2.0 gm of protein per kilogram in which corn-masa was the only protein source. A third post-kwashiorkor boy aged 4 years and 9 months and weighing 15.6 kg was given the basal diet at a level of 1.5 gm per kilogram per day. Nitrogen absorption and retention were measured with each combination for two successive three-day periods as this diet was supplemented step-wise with essential amino acids to match the amino acid pattern of the F.A.O. "reference protein." Initial iso-caloric, iso-proteic milk protein comparison periods were included for the two younger children.

At the levels of protein intake used, the single addition of either tryptophan or lysine did relatively little to restore the negative nitrogen balance occurring with the basal diet, but giving tryptophan and lysine together resulted in markedly increased nitrogen retention in each case. Nitrogen retention was further improved by isoleucine addition but decreased by methionine added at a level intended to match that of the "reference protein." Nitrogen absorption varied from 63 to 81%. The children tended to maintain or gain weight when nitrogen balance was positive and to lose weight slightly during or immediately following periods of negative balance.

The results show that even at an intermediate level of protein intake, the supplementation of vegetable protein with the

appropriate essential amino acids can result in good nitrogen retention by young children.

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