Reprinted from THE JOURNAL OF NUTRITION
Vol. 66, No. 4, December 1958

SUPPLEMENTATION OF CEREAL PROTEINS WITH AMINO ACIDS

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

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(Received for publication May 26, 1958)

The recent report of the F.A.O. Committee on protein requirements reviews the need for taking into consideration the amino acid content of individual proteins and of diets in estimating human protein requirements (F.A.O., '57). It is now well recognized that the relative lack of one or more of the essential amino acids reduces the biological value of food protein (Flodin, '57). Similarly, the correction of this deficiency by a combination of foods whose amino acid content is adequate or by the addition of the missing amino acids in synthetic form has been found in countless animal experiments to result in improved biological value (Flodin, '57). It is also known from studies in experimental animals that the addition of too much of certain of the synthetic amino acids can result in an imbalance which depresses the nutritive value of the protein of the food or diet when fed at relatively low protein levels (Elvehjem, '56; Elvehjem and Harper, '55).

The application of this knowledge to the formulation or supplementation of human diets is greatly hindered by the lack of certainty as to the optimum pattern of amino acids for

[1] Assisted by a grant-in-aid from E. I. du Pont de Nemours and Co., Wilmington, Delaware. INCAP Publication I-111.

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Printed in the United States of America

literature values which subsequently proved too low, the quantities added were actually slightly in excess of the amounts required by the pattern. Only for methionine, however, was this excess observed to have possible significance.

TABLE 1

Amino acid content of corn-masa, corn gluten and the basal diet used in determining the effect of amino acid supplementation on the retention of nitrogen in young children

AMINO ACID	CORN-MASA	CORN GLUTEN	BASAL DIET	FAO "REFERENCE PROTEIN" PATTERN	ADEQUACY OF BASAL DIET	AMOUNT ADDED/GM NITROGEN [1]
		mg amino acid/gm nitrogen			%	*mg*
Arginine	198	184	130	—	—	—
Histidine	177	99	104	—	—	—
Isoleucine	292	245	187	270	69	196
Leucine	607	1152	511	306	—	—
Lysine	200	115	118	270	44	243
Methionine	150	123				
Cystine	83	68	149	270	55	148
Phenylalanine	271	458				
Tyrosine	195	344	376	180	—	—
Threonine	253	232	165	180	91	96
Tryptophan	16	24	12	90	13	148
Valine	282	285	190	360	53	391

[1] Lysine was added in the L-form; all other amino acids in the DL-form. D-isomers were assumed not to be utilized except in the case of methionine.

Corrections were made for the D-form of the amino acids employed by doubling the amount added, except for DL-methionine, which was assumed to be fully utilized and lysine, which was added in the natural form. In these initial studies each experimental combination was fed for a two-day adaptation period followed by a three-day period during which the exact nitrogen intake and output were determined.

In this first study, the relatively high protein intake of 3.0 gm and 100 Cal. per kilogram of body weight was fed throughout. Four balance periods were carried out with a boy, PC-56, 3 years and 7 months old weighing 9.89 kg with a height of 82 cm at the beginning of the trials, and 18 balance periods were completed with PC-57, a boy 4 years and 4 months old weighing 12.0 kg with a height of 86 cm at this time.

RESULTS

Case PC–56. The changes in nitrogen absorption, nitrogen retention and in body weight in case PC-56 are shown in figure 1. Supplementary data are contained in table 2. This child was studied through 4 consecutive nitrogen balance periods. After being in nitrogen equilibrium on the basal diet, the child's nitrogen balance became positive upon the addition to the

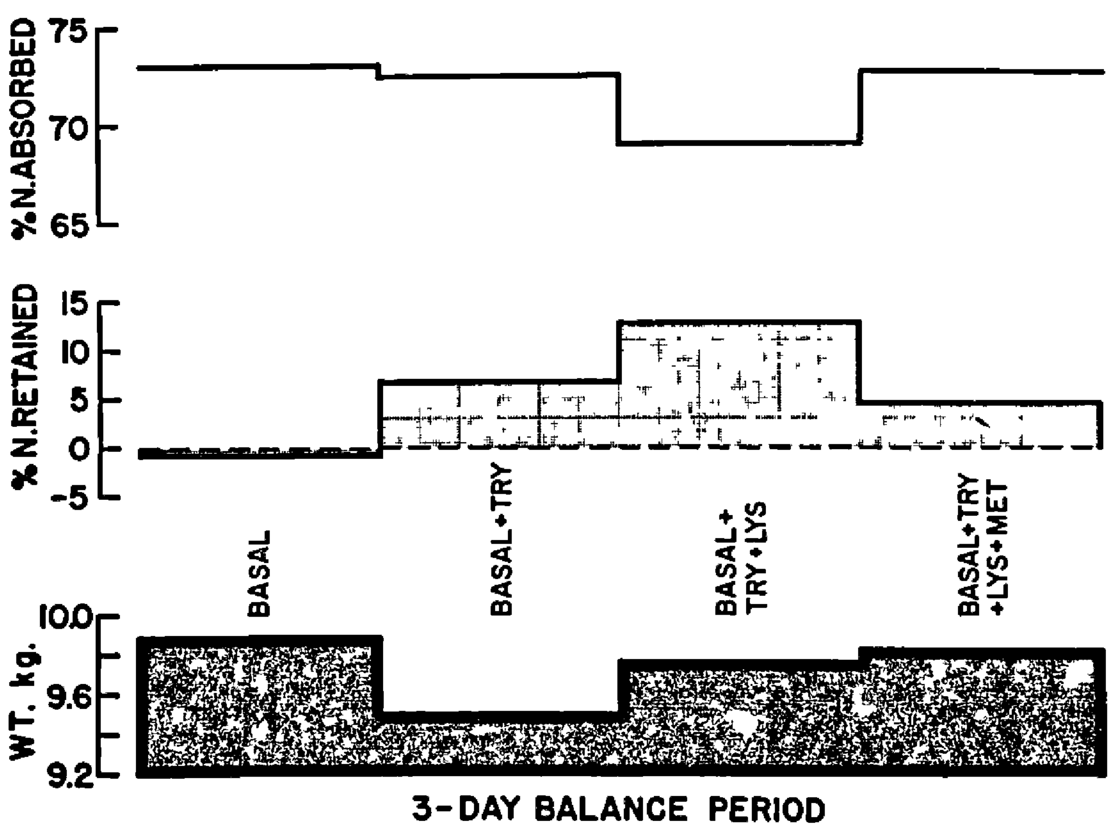

Fig. 1 Effect of amino acid supplementation of a corn-masa basal diet on the per cent nitrogen absorbed and retained by child PC-56 fed approximately 3 gm of protein per kilogram of body weight.

basal diet of 148 mg of DL-tryptophan per gram of nitrogen. The nitrogen balance became more strongly positive when the basal + tryptophan diet was supplemented with 243 mg of L-lysine per gram of nitrogen. The last diet tested, basal + tryptophan + lysine + 148 mg of DL-methionine per gram of nitrogen, did not improve the retention; instead, the methionine supplementation had a negative effect on nitrogen retention. The nitrogen absorption stayed fairly constant throughout the 4 periods. At this point the balance studies had to be discontinued because the child developed chicken pox. During

TABLE 2

Effect of amino acid supplementation of a corn-masa basal diet on the daily intake, excretion and retention of nitrogen by child PC-56

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	449	122	327	73.0	330	— 3	— 0.67	9.89
2	Basal + tryptophan	450	124	326	72.5	296	+ 30	+ 6.67	9.52
3	Basal + tryptophan + lysine	460	143	317	69.0	248	+ 69	+ 12.82	9.77
4	Basal + tryptophan + lysine + methionine	456	124	332	72.8	311	+ 21	+ 4.62	9.83

the basal period the child dropped from 9.89 kg to 9.52 kg. This weight then increased to 9.77 kg during the third period and was 9.83 kg at the end of the experiment.

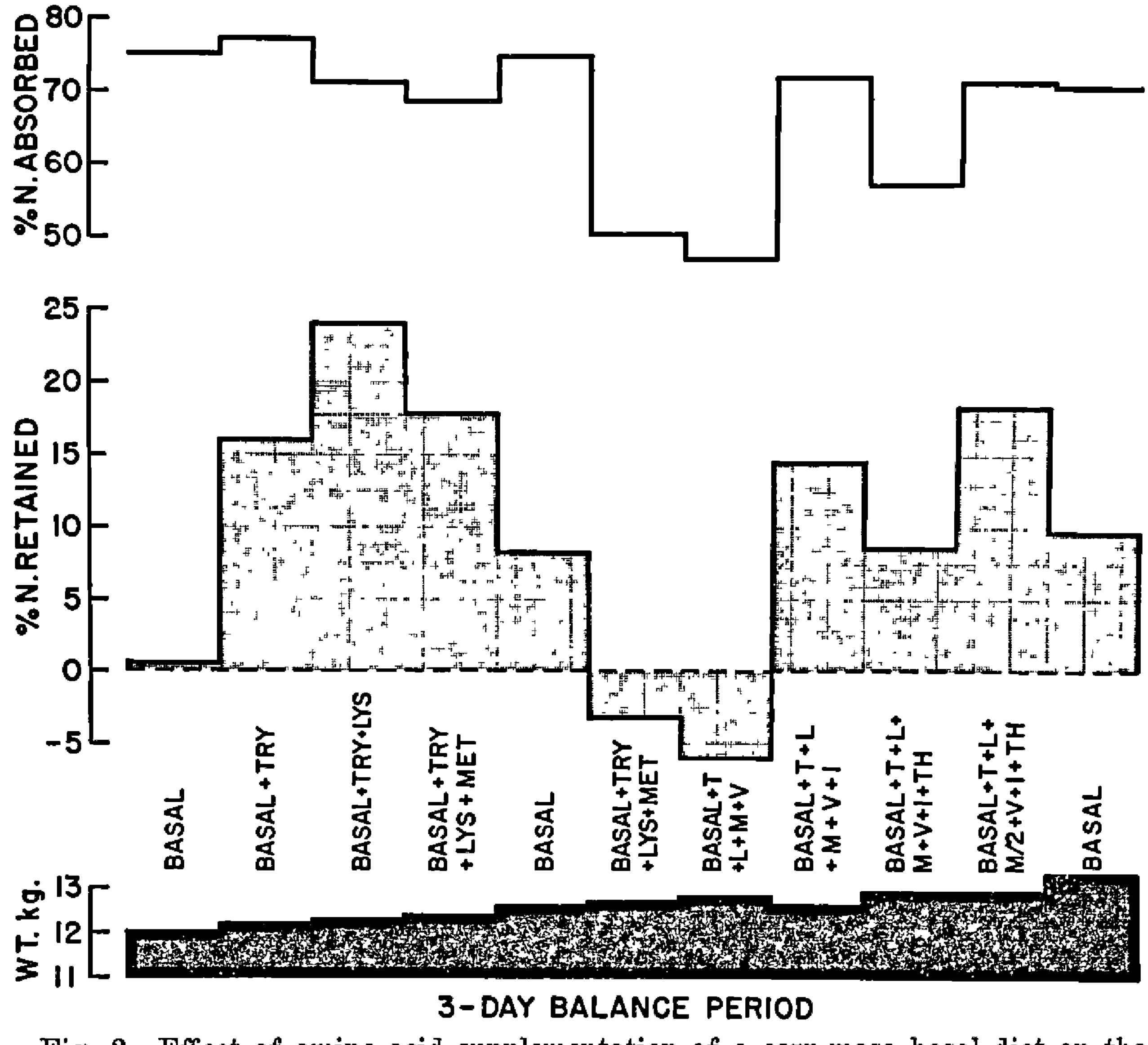

Fig. 2 Effect of amino acid supplementation of a corn-masa basal diet on the per cent nitrogen absorbed and retained by child PC-57 fed approximately 3 gm of protein per kilogram of body weight, Series "A."

Case PC-57. Series A. The same sequence of amino acid additions was tested simultaneously on case PC-57. The results are shown in figure 2. The responses prior to supplementation with methione were qualitatively identical with those of case PC-56, although the responses are quantitatively greater, especially with tryptophan supplementation. The depressing effect of methionine was also apparent in this patient, but to a lesser extent. The child was then returned to the basal diet, and a marked reduction in nitrogen retention occurred. The child was again placed on the basal + tryptophan + lysine diet with 148 mg of DL-methionine per gram of nitrogen added. In this period the nitrogen balance shifted to negative. Furthermore, the nitrogen absorbed, which had been relatively constant up to this point, also decreased sharply.

The addition to the previous diet (basal + tryptophan + lysine + methionine) of DL-valine, the next limiting amino acid, at the rate of 391 mg per gram of nitrogen, made the nitrogen retention even more negative, and the absorption of nitrogen remained poor. On the other hand, the addition of 196 mg of DL-isoleucine per gram of nitrogen to the previous diet (basal + tryptophan + lysine + valine) had a remarkable effect in increasing both nitrogen absorption and retention. The addition of DL-threonine, the least limiting amino acid, at the rate of 96 mg per gram of nitrogen, did not improve nitrogen retention. On the contrary, both absorption and retention decreased in comparison with the previous period.

To investigate the possibility that the level of methionine was too high, a diet with slightly less than half as much added DL-methionine (61 mg per gram of nitrogen) plus the same amount of the other previously tested amino acids was fed. This reduction in the amount of methionine improved nitrogen retention, but not to the level obtained with basal + tryptophan + lysine alone. Feeding only the basal diet again resulted in a decrease in nitrogen retention. Except for the period following the second basal trial, the child continued to gain weight throughout this series of experiments.

Supporting data are given in table 3. In this series of studies with PC-57, the maximum retention of nitrogen, 112 mg per kilogram per day, was obtained on the basal + tryptophan + lysine diet. It was reduced to minus 16 mg per kilogram per day when the same diet + methionine was fed following a basal period and was reduced to its lowest, minus 29 mg per kilogram per day when valine as well as methionine was added to the basal + tryptophan + lysine diet.

TABLE 3

Effect of amino acid supplementation of a corn-masa basal diet on the daily intake, excretion and retention of nitrogen by child PC-57 (Series A)

PERIOD	DIET	NITROGEN INTAKE	NITROGEN IN FECES	NITROGEN ABSORBED	NITROGEN ABSORBED	NITROGEN IN URINE	NITROGEN RETAINED	NITROGEN RETAINED	WEIGHT
		mg/kg/day	*mg/kg/day*	*mg/kg/day*	*% intake*	*mg/kg/day*	*mg/kg/day*	*% intake*	*kg*
A-1	Basal	478	119	359	75.1	356	+ 3	+ 0.6	12.0
2	Basal + tryptophan	464	105	359	77.4	283	+ 76	+ 16.2	12.2
3	Basal + tryptophan + lysine	470	134	336	71.5	224	+ 112	+ 24.0	12.3
4	Basal + tryptophan + lysine + methionine [1]	462	146	316	68.4	233	+ 83	+ 18.1	12.4
5	Basal	471	117	354	75.0	315	+ 39	+ 8.3	12.6
6	Basal + tryptophan + lysine + methionine [1]	451	223	228	50.5	244	− 16	− 3.5	12.7
7	Basal + tryptophan + lysine + methionine [1] + valine	454	241	213	47.0	242	− 29	− 6.2	12.8
8	Basal + tryptophan + lysine + methionine [1] + valine + isoleucine	46[illegible]	128	332	72.2	265	+ 67	+ 14.6	12.6
9	Basal + tryptophan + lysine + methionine [1] + valine + isoleucine + threonine	447	190	[illegible]	57.4	218	+ 39	+ 8.6	12.9
10	Basal + tryptophan + lysine + methionine [2] + valine + isoleucine + threonine	450	129	321	71.5	238	+ 83	+ 18.4	12.9
11	Basal	478	138	340	71.3	294	+ 46	+ 9.7	13.3

[1] DL-Methionine, 0.34%.

[2] DL-Methionine, 0.14%.

Case PC-57. Series B. In order to test the effect of the same amino acids, added in a different sequence, a second series of trials was conducted with PC-57 after keeping him on a milk diet for 12 days. The results are shown in figure 3. Nitrogen retention was measured during a 5-day balance period on milk, followed by two three-day balance periods

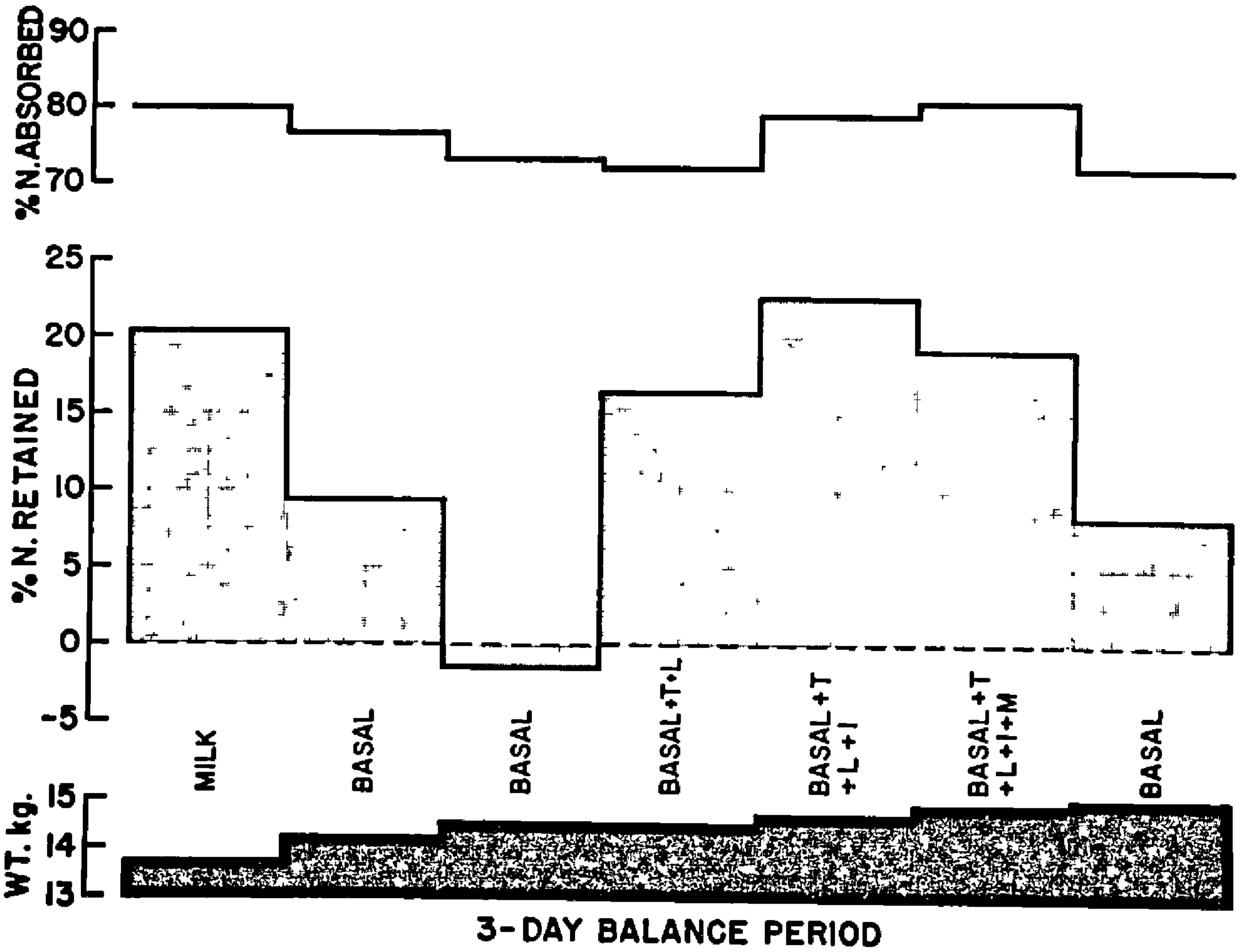

Fig. 3 Effect of amino acid supplementation of a corn-masa basal diet on the per cent nitrogen absorbed and retained by child PC-57 fed approximately 3 gm of protein per kilogram of body weight, Series "B."

during which the basal diet was fed. It can be seen that in the first three day period during which the basal diet was administered, nitrogen retention decreased sharply although the child remained in positive balance. However, during the second three-day period on this same diet, the balance became negative. Since supplementation with tryptophan alone had given positive results each time at this level of protein intake, the basal + tryptophan combination was omitted and the basal +

tryptophan + lysine diet was fed. The balance immediately became strongly positive. The addition of isoleucine improved the retention still further to a level exceeding that of the initial period with milk. The 148 mg of DL-methionine per gram of nitrogen subsequently added to the latter diet had a depressing effect although it was relatively slight. When the child was then returned to the basal diet, nitrogen retention again decreased markedly. Nitrogen absorption remained relatively constant throughout the experiment, and the child continued to gain weight.

From the supporting data given in table 3, it is apparent that the milk diet resulted in a retention of 97 mg per kilogram per day, and a nitrogen absorption of 79.8%. However, a similar absorption of 79.2% on the basal + tryptophan + lysine diet induced a maximum retention of 108 mg of nitrogen per kilogram per day. Upon methionine supplementation, this figure was reduced to 90 mg with no change in nitrogen absorption. On feeding the last basal diet shown in this sequence, the retention decreased to 39 mg, a figure similar to that for the nitrogen retained when the basal diet was fed after the milk period.

DISCUSSION

Although the number of trials is limited and the periods short, the results are remarkably consistent and clear in their implications. Under the experimental conditions, children are sensitive to small changes in the amino acid content of their diets and reflect them in the nitrogen retention and some times in the nitrogen absorption on an isonitrogenous diet. When the nitrogen balance is positive, gain in weight usually occurs. When the first two of the amino acids predicted to be deficient by comparison with the F.A.O. "reference pattern" were added in proportions to approximate the latter, nitrogen retention was markedly improved in each of the three trials. It is noteworthy that the addition of these two amino acids, tryptophan and lysine, in amounts above those calculated to be necessary to reach the level of the next limiting amino acid, produced no detectable imbalance but rather increased nitrogen retention.

TABLE 4

Effect of amino acid supplementation of a corn-masa basal diet on the daily intake, excretion and retention of nitrogen by child PC-57 (Series B)

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*
B-1	Milk [1]	476	96	380	79.8	283	+ 97	+ 20.4	13.7
2	Basal	463	106	357	77.2	314	+ 43	+ 9.4	14.2
3	Basal	453	123	330	73.0	338	− 8	− 1.8	14.5
4	Basal + typtophan + lysine	463	129	334	72.4	258	+ 76	+ 16.5	14.5
5	Basal + tryptophan + lysine + isoleucine	475	99	376	79.2	268	+ 108	+ 22.7	14.7
6	Basal + tryptophan + lysine + isoleucine + methionine	469	90	379	80.8	289	+ 90	+ 19.2	14.9
7	Basal	474	132	342	72.2	303	+ 39	+ 8.2	15.0

[1] Five-day balance period.

However, when methionine, the third limiting amino acid by this criterion, was added, it had a consistently adverse effect on retention. The most obvious explanation is that the "reference pattern" is in error by calling for too much methionine in proportion to the other essential amino acids. Using literature data for estimating the methionine content of the basal diet, the total sulphur-containing amino acids, including the 0.34% methionine supplement, was calculated to be the 270 mg/gm of nitrogen called for by the "reference pattern." Actual analysis subsequently gave a value of 297 mg of methionine + cystine per gram of nitrogen. When the amount of the methionine supplement was reduced to 61 mg per gram of nitrogen, giving a total content of 210 mg of sulphur amino acids per gram of nitrogen, a significant increase in nitrogen retention was observed in comparison with the previous period in which the diet contained 297 mg of these amino acids per gram of nitrogen. The proportion of sulphur amino acids to tryptophan was 3.3 to 1 at the higher level of methionine addition and 2.3 to 1 at the lower, a point of interest since the F.A.O. pattern uses tryptophan, the most deficient of the amino acids in corn, as a reference base. It is also significant that both valine and threonine addition appeared to decrease nitrogen retention, although it should be noted that the levels of these amino acids in the "reference pattern" were very slightly exceeded.

It is a point of major interest and importance that children are immediately sensitive to such small changes in amino acid proportions that they are readily detectable within a single three-day period in nitrogen balance experiments of this type. When the effect of the change on nitrogen retention is large, however, the full effect may not be stabilized in so short a time and hence may influence the magnitude of the response in the succeeding period. This is apparent in the balance studies with PC-57. In series A the combined supplementation with tryptophan, lysine and methionine results in a much lower retention immediately after the second basal period than just before it. In series B retention was much lower in the second of the two consecutive basal periods following the initial trial with milk. In neither series was any infection, gastro-intestinal upset or other possible contributory cause noted. In order to obtain more quantitative estimates of the amounts of amino acid supplements, our further studies employ two consecutive three-day balance periods for each combination tested. That this does not alter the qualitative findings will be demonstrated in subsequent papers in this series.

The data thus far presented emphasize the importance of establishing a proper balance between the essential amino acids if a maximum retention of nitrogen is to be obtained. They also indicate that although the F.A.O. "reference pattern" will not prove ideal for all experimental conditions or physiologic states, this attempt to formulate a provisional pattern is a valuable experimental approach to the problems of amino acid supplementation.

SUMMARY

A simplified basal diet in which corn is the only source of protein was fed to two boys recently recovered from severe protein malnutrition (kwashiorkor) at a level to provide 3 gm of protein and 100 Cal. per kilogram of body weight. Nitrogen balance was determined in a total of 22 three day periods. The children, aged three years and 7 months, and 4 years and 4 months, weighed 9.9 and 12.0 kg and measured 82 and 86 cm, respectively. They continued to gain weight during the progressive supplementation of the basal diet with the amino acids indicated to be deficient by comparison with the amino acid pattern of the F.A.O. "reference protein." The amounts of tryptophan and lysine required to bring the amino acid intake to the reference level resulted in marked improvement in nitrogen retention. This amount of methionine brought about a decrease which could be reversed so that retention became strongly positive by supplementation with isoleucine. Valine and threonine also appeared to have a negative effect on retention at least until after isoleucine had been given. The results show that by the supplementation of a vegetable protein with

the appropriate essential amino acids, good nitrogen retention and satisfactory gain in weight can be obtained, at least with an intake of 3.0 gm of protein and 100 Cal. per kilogram per day in young children.

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