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EXTENDING PROTEIN CONCENTRATES FOR CHILD FEEDING BY ADDITION OF INEXPENSIVE SIMPLE NITROGEN SOURCES

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EXTENDING PROTEIN CONCENTRATES FOR CHILD FEEDING BY ADDITION OF INEXPENSIVE SIMPLE NITROGEN SOURCES

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16. Abstracts A series of studies has been undertaken to determine the extent to which the concentration of essential amino acids in dried skim milk can be extended by the addition of non-specific nitrogen without influencing the nutritional value of the protein. The results of the study, which were conducted in children and young men, are summarized in this report. The non-specific nitrogen source used was a mixture of glycine and diammonium citrate. The results support the conclusion that at any time that it is necessary to extend the "protein" content of milk supplies for the supplementary feeding of population groups, inexpensive non-specific nitrogen sources may be used to an extent of at least 10% without impairing the ability of the milk to meet any normal protein needs.

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INTRODUCTORY STATEMENT

The 1965 FAO/WHO Expert Group on Protein Requirements (1) pointed out that the essential amino acids in most food proteins and particularly those of high biological value are in higher concentration than required by children and young adults. Our review of the published values for the essential amino acid needs of children and young adults supports this view. These data are summarized in Table 1, together with a comparison of the total essential amino acid concentration per gram total protein nitrogen in a selected number of important food proteins. It is also significant that the concentration of the total essential amino acids in the 1957 FAO Provisional Reference Pattern is lower than in most food proteins.

On the basis of these considerations, we postulated that for proteins such as hen's egg, meat, fish protein concentrate, and cow's milk a proportion of the total essential amino acids in these proteins could be isonitrogenously replaced with non-specific nitrogen without altering nitrogen retention and utilization.

We commenced testing the hypothesis in young adult males by feeding a diet in which whole hen's egg protein supplied the major proportion (90%) of total dietary nitrogen. Hen's egg protein was utilized in our initial studies because this protein was adopted by the 1965 FAO/WHO Expert Group as the reference protein (1). A mixture of glycine and diammonium citrate was chosen as the non-specific nitrogen source

because it had been used successfully in other clinical studies and uses the simplest and least expensive available. The results of these studies showed that at about requirement levels the nitrogen of hen's egg protein can be replaced to the extent of at least 30% in young adults without a change in nitrogen retention. For some young adult subjects a 40% substitution (dilution) did not result in an alteration in the utilization of the total nitrogen fed in this way. Since it may have been possible to dilute further the protein given to these subjects, the mean value for the total essential amino acid requirement per gram total N patterened as in egg is still likely to be lower than our published studies suggest (2).

Studies were then extended to beef muscle protein with the conclusion that a diet in which beef protein supplies 90% of the total N can be diluted to the extent of 20-25% without a decrease in the efficiency of utilization of dietary nitrogen by most young men. Unpublished results obtained with fish protein concentrate indicate that the degree of possible dilution with simple and inexpensive non-specific nitrogen sources is also proportional to the relative concentration of the total essential amino acids to total protein N.

With this background, a series of studies were then undertaken with the financial support of the present contract (No. AID/csd-1441) to determine the extent to which the concentration of essential amino acids in dried skim milk can be extended by the addition of non-specific

nitrogen without influencing the nutritional value of the protein. The practical significance of this phase of research is that if dried skim milk shipments could be made to go about 20% further and at lower cost per gram of nitrogen then this would be a significant and immediate contribution to world food protein supplies.

The results of our studies conducted in Children at M. I. T. and INCAP and in young adult men at M. I. T. are summarized in this report.

Table 1

Calculated essential amino acid (g) to total nitrogen (g) ratio in infants, children, and adults from published values on amino acid and nitrogen requirements

Age Group	Total EAA mg/kg	Total N for Growth or Maintenance mg/kg	E/T_N	%EAAN of Total N
Infants (ca. 1 yr)	760[1]	200[2]	3.8	48
Older Children (10-12 yrs)	260[3]	100[4]	2.3	33
Adults	64[5]	46[6]	1.4	16

1. From Holt and Snyderman[3]
2. From Chan and Waterlow[4]
3. From Nakagawa et al.[5]
4. From FAO (Nutrition Studies No. 37, FAO, Rome, 1965) not allowing for growth.[1]
5. Mean for men and women, from FAO[6]
6. From Young and Scrimshaw[7]

METHODS

The maximum extent to which skim milk protein fed to children can be isonitrogenously replaced with non-specific nitrogen was evaluated by methods which have been described in detail in earlier progress reports, AID Contract No. AID/csd-1441, and in previous publications (2, 7-9). Therefore, the methods will not be described in detail in this report.

EXPERIMENTAL AND RESULTS

A. Studies of the effects of milk protein dilution in children (M. I. T.)

Studies on the effect of dilution of skim milk protein were commenced on fourteen mentally retarded children. Of these fourteen children, seven were found to be unsatisfactory for study and returned within two weeks of admission to the M. I. T. Clinical Center, to their respective State Schools.

Details of experimental design, diet, and analytical procedures were previously described in the 1967 Annual Progress Report on this project and are not repeated here.

Details for the seven children who were studied during the entire experimental period are given in Table 2, on Page 5.

Table 2

Age and weight of children and experimental design used for studies of milk protein dilution[1]

Subject	Age (yrs.)	Initial Wt. (lb.)	Final Wt. (lb.)	Days on Baseline Diet	Days on Dilution
P.K.	9	61.5	58.5	18	20
W.M.	6	33.0	36.0	26	83
C.R.	5	33.0	35.0	25	32
R.O'C.	4	34.0	36.0	33	32
B.L.	5	41.0	43.0	29	40
S.D.	9	35.0	37.0	25	12
J.W.	5	22.0	23.5	40	30

1. Dilution at 20% level.

Results on urinary nitrogen excretion for these subjects during the baseline and milk dilution periods are summarized in Table 3.

Table 3

Effects of dilution of skim milk protein with glycine and diammonium citrate on urinary nitrogen excretion by children

Subject[1]	Dilution of Protein, %	
	0	20
P.K.	2.14 ± 0.24	2.09 ± 0.59
W.M.	0.77 ± 0.14	0.70 ± 0.12
C.R.	0.81	0.81
R.O'C.	1.03 ± 0.1	0.92 ± 0.88
B.L.	1.17 ± 0.04	1.08 ± 0.2
S.D.	0.86	0.89
J.W.	0.66	0.42

1. All subjects fed milk protein as the major protein source (90% total dietary N) at a level equivalent to 0.45g protein/kg body wt./day. Figures are mean ± std. dev. for daily urinary nitrogen excretion (g/day) for periods of four or more cons. days.

Thus, using nitrogen excretion as the parameter for assessing the effect of a 20% dilution of cow's milk protein with non-specific nitrogen it appears that this level of dilution in school-age children does not alter the efficiencies of dietary nitrogen utilization. It may, therefore, be concluded that cow's milk protein, when fed at 0.45 g protein/kg body wt., contains about 20% essential amino acid N in excess of the essential amino acid requirements of these children. These results agree well with those described below for young adult men. As revealed by clinical examination and routine clinical chemistries, all children maintained good health throughout the experimental period.

B. Studies of the effects of milk protein dilution in children (INCAP)

On the basis of our preliminary observations at M.I.T. with mentally retarded school-age children and from our findings in young adult men, a series of studies were conducted at INCAP in children recovering from severe protein-calorie malnutrition.

In the first series of studies the following objectives were pursued:

1. To study, in protein depleted children, the utilization of milk whose essential nitrogen was diluted 10% with glycine and diammonium citrate (10% diluted milk protein). The criteria used were nitrogen balance complemented by clinical evaluation of the children. Theoretically, by the use of protein depleted children, differences

could be detected between proteins of similar quality which in fully repleted individuals would go unnoticed. The sensitivity of the depleted child to the availability of essential nitrogen should be greater due to the acute and specific need of essential nitrogen for the rapid protein synthesis of young depleted organisms.

2. To test the supplementary value of milk protein versus "10% diluted milk protein", when added to a typical diet consumed by pre-school children in Central America. The protein in this typical diet is known to be of poor quality, mainly because of deficiencies in lysine, tryptophan and methionine.

Objective 1. The design of the study, involving four children during the early phase of recovery from severe protein-calorie malnutrition(PCM) the methods followed and detailed results were described in detail in the "February 1969 Annual Progress Report." The plan of the study is shown in Fig. 1.

Summary of Results: Table 4 summarizes the average values, obtained with 3 consecutive three-day N balance periods for the nitrogen absorbed and retained by each child with each diet;and also presented in the table are the mean results for the four children during each of the three experimental diet periods.

No differences were detected by the nitrogen balance technique between milk and "milk diluted 10%" (i.e. replaced 10% with non-specific nitrogen) in protein depleted children. The constancy of urinary nitrogen

excretions in these children brings further evidence to the similar behaviour of the children during the three study periods.

Apparent nitrogen absorption varies primarily due to fecal nitrogen excretion and this factor influences the apparent total nitrogen retention more than changes in urinary nitrogen. These variations are due to individual patterns of fecal nitrogen excretion during the early recovery phase of malnutrition in each child and are not due to variations in the diet. The use of children who are still protein depleted for the evaluation of nitrogen sources has this limitation, particularly when nitrogen intake is low. In this case, changes in fecal endogenous nitrogen that are known to occur in children with PCM can account for a large proportion of the fecal nitrogen. The variation in apparent nitrogen absorption and retention in these children, therefore, is greater than that expected in fully recovered ones.

Clinical Evaluation

The four children studied did not show any clinical alteration from either of these diets. However, the rate of weight gain was slightly reduced in the four children while they were receiving 1.0g. Prot/kg/day. This slower weight gain was not accentuated during the period on "milk diluted 10%". In a similar fashion, creatinine excretion did not rise as rapidly as in children who are maintained on high protein intakes during this phase of recovery. Total serum proteins decreased in two of the four children, particularly in child PC196 who remained

close to nitrogen equilibrium during the study. These biochemical changes were not further accentuated during the period on "milk diluted 10%".

This study led to the conclusion that a 10% dilution of cow's milk protein, by replacement with non-essential nitrogen, does not reduce the protein quality of milk for young children when it is the sole source of dietary protein.

Objective 2.

Background and Design

The second aim of these investigations was carried out by determining, by means of nitrogen balances, the supplementary value of "10% diluted milk protein" when compared to whole milk when fed as dietary supplement to a diet which resembled the rural diet of poor Central American populations, based on corn and black beans.

The rational for this question was that when milk was the only source of protein the dilution of its essential nitrogen still left an excellent quality protein: however, dilution of its essential nitrogen could conceivably reduce the availability of the "relative excess" of essential nitrogen for supplementation of poor quality protein diets.

The research protocol consisted of feeding a corn and black bean diet alone followed by this diet plus a supplement of milk and "10% diluted milk protein" in consecutive randomized periods to six children who were fully recovered from malnutrition and whose ages ranged from 1-7/12 yr. and 3-4/12 yr.

Materials and Methods

The characteristics of the children who were the experimental subjects is shown in Table 5. Child PC-201 was started on this study but had to be discontinued after 18 days because of progressive intolerance to the corn and bean diet.

The study was started by placing the children on a corn and black bean diet which was reconstituted from a homogenized and lyophylized powder prepared especially for this study at INCAP. The source of protein was 20% from beans and 80% from corn.

The children received 1.6 g Prot/kg/day from this mixture. Calories were added in the form of carbohydrates and fat to supply 100 Cal./kg/day; 20% of the total calories were in the form of fat. Vitamin and mineral supplements were given throughout the study to supply the recommended allowances of these elements. The children were kept on this diet for 10 days. The first four days were of adaptation and the next 6 days were divided in two 3 day nitrogen balance periods, performed by the standard techniques at use at INCAP. This initial dietary period was followed by a second and third dietary period which consisted of the corn and bean at the same protein level of 1.6 g/kg/day plus either whole cow's milk or "10% diluted milk protein" to provide 0.4 g/Prot./kg/day. Calories, fat, vitamins and minerals were kept constant at the level of the first period of study. Each dietary period lasted 10 days divided in four days of adaptation

and two 3 day nitrogen balances. Whole milk preceded "10% diluted milk protein" as a supplement in 3 children and followed it in 3 other children. The only variables in the study were, therefore, the level and source of protein. With the milk supplemented diets the only variable was the source of the protein supplement, either whole milk or "10% diluted milk protein".

Results: In each of 3 children (PC-196, 198 and 199), 3 balance periods were performed while in the corn and bean diet alone to establish further if the N balance results of two 3 days collection periods of this diet were improved by a third period. The differences were not significant ($\overline{X}$ N retention with two 3 day periods = 9.6%). Consequently the other 3 children, and throughout the study, two three day balance periods were used.

Tables 6 to 11 show the data on each individual child with regard to nitrogen balance. It is readily seen that both nitrogen absorbed and nitrogen retained increased in each child as milk was added to the rural diet. There was no difference whether the milk added was whole milk or its essential nitrogen had been diluted 10%. There is only one nitrogen balance with rural diet alone (1st balance of child PC-196) whose nitrogen absorbed and nitrogen retained overlap with the results obtained with rural diets supplemented with either whole milk or diluted milk.

Table 12 summarizes the overall results for the 6 children studied. It can be observed that the addition of 0.4 g of milk protein per kilo per day, whether diluted or not, to a corn and black bean diet fed at a level

of 1.6 g protein/kg/day, significantly improves both nitrogen absorption and nitrogen retention.

In relation to the overall goals of this project, it was encouraging that there was no difference in the supplemental effect of milk protein when given as whole milk or milk whose essential nitrogen is diluted 10%.

These results lead to the conclusion that the supplementary value of milk whose essential amino acid nitrogen is diluted 10% with non-essential nitrogen still has enough essential amino acid nitrogen to serve as a supplement to the rural diet tested. These results establish the theoretical principle of the possibility of diluting essential amino acid nitrogen to extend the availability of essential nitrogen sources for supplementation of poor diets.

For essential amino acid nitrogen dilution to be of public health importance in the sense of significantly extending available food protein sources as well as making the process economically feasible, it was considered necessary to evaluate the effects of a further dilution of milk with non essential nitrogen, the extent of 20 or even 30%, and worthwhile to evaluate the effects of addition of the limiting amino acids (specifically methionine) to the diluted milk, once its supplementary value was reduced by the non-specific nitrogen dilution. The results of these studies are described under Objectives 3 and 4.

Objectives 3 and 4.

On the basis of the above results indicating that a 10% dilution of cow's milk protein with non-specific nitrogen did not reduce either the

nutritional value of this protein for pre-school children as its supplemental value when given in conjunction with a diet of corn and beans the following additional objectives have been pursued in pre-school children:

Objective 3:

What is the maximum reduction in the total essential nitrogen concentration of milk protein which can be achieved before a reduction of its nutritive value for pre-school age children ("safe maximal dilution").

Objective 4:

Whether by the addition of the specific limiting amino acids to milk, its total essential nitrogen concentration can be reduced beyond that of "safe maximal dilution" without losing some of its nutritive value for pre-school children.

Material and Methods (Objectives 3 and 4)

Eight pre-school age children who had been previously severely malnourished but who were fully recovered after at least three months of therapy at INCAP's Clinical Center, were the subjects in this study. One of them (PC-226) was studied in two occasions with two different protocols. All of the children fulfilled the criteria set at this Center to consider them completely recovered, mainly: a) Weight for height above 95%; b) Creatinine height index above 0.90; c) Normal serum proteins and hematological condition; and d) Absence of any signs of malnutrition or infection.

All of the children were receiving, before the study, a milk casein diet providing 3 g. of protein and 100 calories/kg/day, plus a vitamin-mineral supplement. Twenty percent of the calories were provided by vegetable fat. During the study the children received a diet which provided a constant amount of nitrogen equivalent to 1 g. of protein per kilo, the rest of the nutrient intake remaining as it was before the experiment. The only variable throughout the study was the source of nitrogen.

Experiment 3 designed for Objective 3: Five children took part in this study. Each child received four different diets, remaining on each diet for a period of 10 days. The 10 day period was divided into a 4 day adaptation period and two 3 day nitrogen balance periods, in that order. The next diet was started immediately after the second balance on the previous diet was finished. The sequence of the diets tested was the following: Milk, milk to which glycine was added to provide an amount of nitrogen equivalent to 20% of the milk nitrogen (milk diluted 20%), milk diluted 25%, and milk diluted 30%.

Experiment 4 designed for Objective 4: Four children took part in this study, in which four diets were tested. Each diet was administered for 10 days divided into adaptation and N balance periods as previously described. The diets were the following: milk, milk diluted 30%, milk diluted 40%, and milk diluted 40% but with methionine added to provide a concentration equal to that present in the 30% diluted milk. The

first and second diets were always milk and milk diluted 30%. The last two diets were administered in alternate sequences as follows: two children received 40% diluted milk, followed by 40% diluted milk + methionine, followed by 40% diluted milk. Two other children received 40% diluted + methionine, followed by 40% diluted, followed by 40% diluted + methionine.

Results (Objectives 3 and 4)

Appendix Tables 1 to 9 present the data obtained in each individual child studied during these two series of experiments (Experiments 3 and 4). Figure 2 shows the mean N retention (mg/kg/day) obtained in Experiment 3. Although there appears to be a tendency to retain less nitrogen with diluted milk, the differences are not statistically significant, and there is a slight increase in N retention with 30% dilution in relation to 25% dilution.

Figure 3 shows the mean N retention (mg/kg/day) for Experiment 4. These results show that there is a decrease in N retention with 30% diluted milk, but that with 40% diluted milk N retention rose again to levels even superior to those obtained with milk.

The fact that in both experiments there was a tendency for N retention to decrease during the initial part of the study, and to rise in the later parts of the experiment led us to consider the effect of the sequence of the experimental diets. Comparing the results obtained with milk diluted 40% with or without methionine corresponding to the

third period against those obtained when 40% diluted milk with or without methionine was administered as the 4th and 5th treatment, the following results are obtained:

	Average ± S.E. N Retention (mg/kg/day)		
	3rd Treatment	4th Treatment	5th Treatment
Milk Dilution 40%	16.0 ± 3	51.2 ± 5	56.2 ± 8
" " 40%Meth.	20.5 ± 9	54.8 ± 5	55.0 ± 4

These results are expressed in graphic form in Fig. 4. It is evident that N retention rose after 10 days when the children were kept on 40% diluted milk with or without methionine. The addition of methionine did not produce a significant rise in N retention, although it produced slightly higher retentions, in the third and fourth periods.

The results of these studies (Exp. 3 and 4) indicate that with 20% dilution milk does decrease its protein quality, although slightly, and that further dilution decreases its quality even further. This conclusion is reached when the average N retention in each experiment is expressed in % of the N retention obtained with the same set of children given the undiluted. Fig. 5 presents the overall results of these experiments and reveals a linear relationship between level of dilution and the degree of total dietary N retention. An average for each percentage of milk dilution beyond 15%, N retention decreases by 2.3%. These results are highly significant (r=0.97).

An additional observation may be made from the above analysis. If the N balance results obtained when the 40% diluted milk with or

without methionine, was administered at the 3rd dietary period an effect of supplementary methionine is clearly evident and suggests that N retention values is improved to a level close to that obtained with 30% diluted milk (i.e., to the level to which the methionine concentration was adjusted). Thus, this further strongly suggests that under the experimental conditions methionine was the limiting essential amino acid.

A further comment regarding the design of Exp. 3 and 4 should be made. In our initial studies with protein-depleted children, milk diluted 10% with glycine appeared to have essentially the same quality as milk. In Exp. 3 and 4 the children were fully protein repleted and a decrease in N retention was detected after only a 20% dilution. Thus, the question remains as to whether a 10% dilution in fully repleted children produces a detectable reduction in N retention. However, the studies with fully repleted mentally retarded children would indicate that a 10% dilution of milk protein would not reduce the protein quality of milk protein for normal pre-school children.

The critical importance of the state of nutrition of the subjects is also brought out in the results of Exp. 3 and 4. The fact that 40% diluted milk produced highly satisfactory N retention in these children when it was administered after a period when N retention had decreased to levels which were below that required for adequate growth suggests that at that time the children were slightly nitrogen depleted and had

adapted by an increase in the efficiency of dietary N utilization. This hypothesis is strengthened by the fact that in fully repleted children 1.0 to 1.2 g of milk protein per kilo per day are required to produce biochemical normality and N retention compatible with growth.

Finally, the effects of milk dilution were tested at a very critical N intake level, that is, at about the levels of milk protein requirements. Here, any decrease in milk protein quality should be easier to detect.

From these studies with preschool children it appears that the "safe maximal dilution" of essential nitrogen in milk is greater than 10%. Also, it appears that the specific addition of the most limiting amino acid in milk allows for further dilution of its total essential nitrogen beyond the "safe maximal dilution". The level to which dilution can be safely increased with methionine addition remains to be determined.

C. Studies in young adult men

The effects of milk protein dilution were first evaluated in young adult men to provide a basis for the adequate design and interpretation of studies conducted in children.

1. First series

The results of the first series of studies in young adult men have already been published and a summary of the findings are given as follows:

Skim milk protein was replaced isonitrogenously by glycine and diammonium citrate, or by a mixture of nonessential amino acids in the test diet of 21 male college students; the effect of this replacement on urinary nitrogen and sulfur excretion and on fasting plasma amino acid concentration was studied. The subjects were fed a constant nitrogen intake equivalent to 0.38 g protein/kg body weight. Skim milk protein furnished 90% of the daily protein; oatmeal furnished the remainder. In Exp. 1 and 2, glycine and diammonium citrate replaced 20 and 25% of total dietary nitrogen, respectively. In the third experiment, a mixture of nonessential amino acids replaced 25% of dietary nitrogen. The 20% replacement with glycine and diammonium citrate did not increase urinary nitrogen excretion in any of the seven subjects, but 3 of the 7 showed lower urinary nitrogen excretion after return to the basal diet. A 25% replacement with glycine and diammonium citrate increased urinary nitrogen excretion in 3 of 7 subjects and 4 subjects showed lower nitrogen output during the final basal period. The 25% replacement with nonessential amino acids did not change urinary nitrogen excretion but 3 of 10 subjects showed a variable decrease in nitrogen excretion during the second basal period. As a replacement for 25% of total dietary protein, nonessential amino acids appeared a more effective nonspecific nitrogen source than glycine and diammonium citrate. Fasting plasma amino acid levels were unchanged during the experimental periods.

From these findings it is concluded that when skim milk protein provides essentially the sole source of dietary protein, it may be diluted by a minimum of 20% without a change in its nutritive value.

2. Second Series

The above findings with young adult men raised a number of fundamental questions in regard to the evaluation of protein quality for human nutrition. In a limited series of rat-feeding studies, we found that cow's milk protein could not be partially replaced with non-specific nitrogen in contrast to the findings with young adult men, and subsequently confirmed in pre-school and school-age children. (A summary of the rat experiments is given in Appendix 2.)

Furthermore, extensive studies in experimental animals indicates that the NPU (Net Protein Utilization) of cow's milk is about 75 and this compares with values for egg of about 95-100. If these quantitative relationships also apply to adult man, it would not be anticipated that a 25% dilution of skim milk protein with non-specific nitrogen would markedly reduce its protein quality. However, as discussed above, this was not found to be so and based on our dilution studies the nutritive quality of cow's milk protein appeared to approach that of egg protein. It was, therefore, important to further consider this question in order to support the interpretation and implications drawn from the results of the milk dilution studies. There is also a serious lack of data on the relative nutritional quality of cow's milk for human

nutrition and thus the studies described below also add useful knowledge in this area as well as more immediately providing a basis for evaluation of the extent to which cow's milk protein can be replaced by a non-specific nitrogen source.

Two studies were conducted. In the first, 8 healthy young adult male M.I.T. students participated in an experiment designed to compare the nutritive value of cow's milk protein, cow's milk protein supplemented with L-methionine and reference protein (whole hen's egg protein). The consecutive dietary periods were as follows: (a) an initial two-day period on a protein-free diet to aid rapid adaptation to the low-protein test diets; (b) a 15-day period of giving cow's milk protein at 0.27 g protein/kg; (c) a 15-day period of giving cow's milk protein supplemented with L-methionine. The level of methionine supplementation was equivalent to 2.08% of the total cow's milk protein (Nx6.25) intake; (d) a 15-day period of giving hen's egg protein at a level of 0.27 g protein/kg body wt. per day. The first five days of each 15-day period were taken as adaptation days and the results of the studies based on the final 10 days of each test period.

The second study, with 6 young male adult subjects, compared the protein nutritional quality of cow's milk and its major protein components, casein and lactalbumin. The design of this study was essentially identical to the one above except that dried skim milk, casein and lactalbumin were given at a level to supply 0.27 g protein (Nx6.25) per kg body wt. during consecutive 15-day test periods, respectively.

Effects of methionine supplementation and the nutritive value of cow's milk protein: the nitrogen balance data obtained for each subject in the first study are summarized in Table 13, and the derived values for the digestibility, biological value (BV) and net protein utilization (NPU) of cow's milk protein, with and without methionine supplementation, compared with reference protein (whole hen's egg), are summarized in Table 14.

These results indicate that methionine supplementation did not improve the nutritional value of DSM and, further, that the BV of DSM does not differ significantly ($P > 0.1$) from that of egg protein.

Comparison of the nutritive value of cow's milk protein: the nitrogen balance data obtained for each subject in the second study of this series are summarized in Table 15 and the derived values for the digestibility, biological value (BV) and net protein utilization (NPU) of cow's milk protein, casein and lactalbumin are summarized in Table 16.

The results of this experiment show that the BV of DSM is not significantly different from the BV of lactalbumin. The comparison between DSM and casein is rather more difficult because one subject (AV, see Table 15) showed an improved N balance when given casein, as compared with that when DSM was given as the test protein in comparison with the other five subjects whose N balance deteriorated when casein was given. The reason for this discrepancy appears to

be partly due to the higher fecal N output of subject AV when DSM was consumed, thus, resulting in a more negative N balance. Whilst we have no explanation for this, it appears likely that fecal N output was unusually high for this subject during the period of consuming the DSM. We therefore consider it valid to evaluate the derived values (as shown in Table 16) for the nutritive value of DSM versus casein on the basis of the five subjects (i.e. excluding subject AV). In this case, the BV and NPU of casein is significantly ($P<0.01$) lower than that of DSM proteins for young adult men.

From these studies it is clear that even at relatively low levels of protein intake the nutritional quality of cow's milk protein approaches that of egg for the adult human. The lactalbumin component of the cow's milk protein appears to fully compensate for the relatively poor quality of casein for adult man. In view of these observations and because methionine supplementation has no effect in improving the protein quality of cow's milk protein when given at relatively low-level of protein intake, it would be anticipated that a certain level of dilution of this protein could be achieved without a decrease in its capacity to meet human protein and amino acid needs. Thus, the conclusions drawn from the results of the dilution studies find support from these observations and further indicate that the concentration of the total essential amino acids in cow's milk protein is in excess of the needs of the adult for adequate protein nutrition.

3. Third Series

A study with young adult men was conducted to determine whether the supplementary effects of milk protein, when added to an all vegetable protein diet, was reduced by first replacing 20% of the total milk protein N with glycine and diammonium citrate. This study paralleled the one described above with pre-school children given a diet of corn and beans to which milk or 10% diluted milk was added.

The design of the experiment was as follows: 6 young adult M. I. T. students participated in this study. The experiment consisted of three 15-day experimental diet periods. The first involved feeding a low-protein basal diet (about 0.2 g protein/kg/day) with wheat providing 85% and chick pea 15% of the total dietary protein. During the subsequent and consecutive 15-day diet period 0.2 g skim milk protein/kg was added to the basal diet and during the third and final 15-day period the 20% diluted milk protein was used for supplementing the basal diet. The order of these latter two dietary phases was randomized for the 6 subjects studied. The first five-day period of each dietary phase was considered as an adaptation phase and the final 10-day period was used for calculation of the results. The details of methods and analyses were described in our previous publications.

Results for urinary nitrogen excretion and for nitrogen balance are summarized in Tables 17 and 18, respectively.

Supplementation of the all-vegetable protein diet with skim milk protein significantly increased ($P<0.001$) N retention, as we had anticipated. However, the improvement in N balance by the addition of 0.2 g milk protein/kg to the basal, low-protein diet, was greater for the undiluted than for the 20% diluted milk. This is reflected by the lower Biological Value ($P<0.05$) for the diet containing the diluted skim milk compared with the diet containing the undiluted skim milk (Table 19).

From these results it appears that a 20% dilution of skim milk protein reduces its full capacity to improve N retention on an all-vegetable low-protein diet. It is not know whether a 10% to 15% dilution of the skim milk protein would reduce the supplementary value of milk protein. It should also be pointed out that in these experiments one half of the total protein intake on the supplemented diet was provided by the supplementary skim milk protein. This raises a further point as to whether a 20% dilution of milk protein reduces the supplemental effect when the basal diet is less-deficiet but still supplies an inadequate daily protein intake. The answers to these questions can only be obtained from further detailed studies.

SUMMARY AND CONCLUSIONS

A. Comparison of the essential amino acid and total nitrogen requirements of children and adults, as reported in the scientific literature, with the concentration of essential amino acids found in milk and other good protein sources of animal origin leads to the conclusion that for healthy individuals beyond infancy the concentration is higher than the physiological requirement. Under these circumstances, it should be possible to replace part of the nitrogen of these protein sources with nitrogen from any utilizable source, including simple and relatively inexpensive nitrogen containing compounds such as glycine, glutamic acid and ammonium citrate.

B. Nitrogen balance studies with young adults at M. I. T. fed milk as a sole nitrogen source indicated that at least 20%, and in some subjects greater than 25%, of the nitrogen of milk could be so placed without affecting nitrogen retention. These findings and conclusions were supported by additional studies showing that the biological value of cow's milk protein and egg protein (reference protein) were nearly identical for young adult men. These latter observations contrast with those obtained with the rat and further caution against a broad extrapolation of findings made with experimental animals in assessing the nutritional quality of dietary protein for man.

C. In order to allow a margin of safety in the studies with children, a 20% dilution was employed with seven children aged four to nine years

in studies at M.I.T. with similar results. While these results indicated that a 20% dilution is effective with children, the number of subjects was too small to give much indication of the extent of individual variation. For this reason a still conservative dilution, 10% was recommended for initial studies of nitrogen balance during the repletion of malnourished and younger children in the Institute of Nutrition of Central America and Panama. Once again there were no differences in nitrogen retention between periods with the milk formula diluted or undiluted with non-specific nitrogen. Studies were then conducted in recovered children to determine the maximum extent to which milk protein could be replaced by non-specific nitrogen. The nutritional value of milk protein, when it provided the sole source of dietary protein was reduced when the dilution with non-specific nitrogen is greater than 10% of the total milk protein N. Additional studies of methionine supplementation indicated that the concentration of this amino acid in milk protein limited the extent to which the protein could be replaced by the non-specific nitrogen source without affecting dietary protein quality. The maximum extent to which cow's milk protein, when adequately supplemented with methionine, can be diluted by non-specific nitrogen without a change in protein quality for child feeding remains to be determined.

These results give a reasonable assurance that the nutritional contribution of milk as a sole source of protein can be extended to a

at least 10% by addition of simple and relatively inexpensive nitrogen sources.

D. In two studies, one with protein-depleted children and the other with young adult men, were conducted to determine whether a dilution of milk protein by non-specific nitrogen influenced the supplementary value of the food protein when added to a basal, all-vegetable protein diet. For children given a diet based on corn and beans, 10% diluted milk gave the same adequate supplementary response as undiluted milk. In young adult men given a low-protein diet based on wheat and chick pea, the supplementary value of milk which provided half of the total daily protein intake on the supplemented diet, was reduced by a 20% dilution with non-specific nitrogen.

E. The overall conclusion is that at any time that there is a need to extend the "protein" content of milk supplies for the supplementary feeding of population groups there is a strong experimental basis for doing so with non-specific nitrogen sources to a level of 10% without impairing its ability to meet protein needs for any of the normal uses of these milk supplies. Further work would be required to determine how much more than a 10% dilution could be achieved for various circumstances.

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TABLE 4

Average nitrogen intake, absorption (%) and retention (%) of each child studied for the effects of a 10% dilution of milk protein with non-specific nitrogen

Child No.	Study Period	Protein Source	N Intake (mg/kg/day)	N Absorbed (%)	N Retained (%)
PC-193	1	Milk	158	72.5	20.4
	2	Milk-Dil. 10%	154	81.1	33.1
	3	Milk	148	68.2	24.4
PC-194	1	Milk	165	77.4	23.5
	2	Milk-Dil. 10%	160	74.5	18.9
	3	Milk	158	72.7	21.2
PC-196	1	Milk	160	73.4	-5.2
	2	Milk-Dil. 10%	153	67.3	11.3
	3	Milk	154	59.2	7.7
PC-197	1	Milk	151	85.7	28.5
	2	Milk-Dil. 10%	151	86.6	25.0
	3	Milk	153	86.4	37.1
Mean	1	Milk	158.4	77.2	16.8
	2	Milk-Dil. 10%	155.2	77.1	22.1
	3	Milk	152.9	77.2	22.6

TABLE 5

Characteristics of children studied for the effects of dilution of skim milk when used as a supplement to a corn and bean diet

Child	Age (m)	Months in Clinical Center	Weight for Height %	Height for age %	Creatinine Height Index %	Total Serum Proteins gm
PC-196	40	7	110	87	1.03	7.60
PC-198	42	5	108	94	1.03	7.70
PC-199	28	2	104	85	1.00	6.00
PC-200	19	2	98	92	0.94	6.20
PC-202	24	3	93	84	0.82	6.40
PC-204	27	2	97	89	0.94	6.30

TABLE 6

CHILD PC-196

Age: 3 years 6 months

Height: 86 cms.

Diet	Weight	N. Intake mg/Kg/d		N. Absorbed		N. Retained	
		Planned	Actual	% of intake	mg/Kg/d	% of intake	mg/Kg/d
Rural	13.84	256	256	68	173	23	59
	13.95	256	252	56	140	8	21
	13.90	256	253	62	157	10	27
Rural + Milk	14.00	320	319	63	202	25	80
	14.06	320	284	66	188	18	50
Rural + Dil. Milk	14.34	320	314	74	234	33	104
	14.58	320	314	70	221	26	81

TABLE 7

CHILD PC-198

Age: 3 years 6 months

Height: 93 cms.

Diet	Weight	N. intake mg/kg/d. Planned	N. intake mg/kg/d. Actual	N. Absorbed % of intake	N. Absorbed mg/Kg/d	N. Retained % of intake	N. Retained mg/Kg/d
Rural	14.68	256	257	66	170	12	30
	14.86	256	254	70	177	11	28
	15.08	256	234	54	126	-13	-29
Rural + Dil. Milk	15.14	320	314	80	253	23	74
	15.37	320	280	74	209	11	31
Rural + Milk	15.54	320	310	77	239	35	107
	15.60	320	301	73	221	26	78

TABLE 8

CHILD PC-199

Age: 2 years 4 months

Height: 74 cms.

Diet	Weight	N. intake mg/Kg/d		N. absorbed		N. Retained	
		Planned	Actual	% of intake	mg/Kg/d	% of intake	mg/Kg/d
Rural	10.83	256	254	57	144	4	10
	10.89	256	251	57	144	8	19
	10.94	256	249	65	163	17	42
Rural + Milk	11.34	320	312	71	222	30	95
	11.46	320	317	76	241	29	90
Rural + Dil. Milk	11.62	320	317	70	220	23	72
	12.02	320	328	71	233	26	83

TABLE 9

CHILD PC-200

Age: 1 year 8 months

Height: 76 cms.

Diet	Weight	N. Intake mg/Kg/d		N. Absorbed		N. Retained	
		Planned	Actual	% of Intake	mg/Kg/d	% of Intake	mg/Kg/d
Rural	9.98	256	255	70	179	12	32
	9.98	256	260	56	146	12	32
Rural + Milk	10.15	320	325	72	234	24	79
	10.38	320	316	74	233	27	84
Rural + Dil. Milk	10.54	320	305	74	227	30	90
	10.60	320	296	78	232	29	86.

TABLE 10

CHILD PC-202

Age: 2 years 0 months

Height: 70 cms.

Diet	Weight	N. Intake mg/Kg/d		N. Absorbed		N. Retained	
		Planned	Actual	% of Intake	mg/Kg/d	% of intake	mg/Kg/d
Rural	8.05	256	249	54	136	-16	-40
	8.10	256	248	51	127	-15	-38
Rural + Dil. Milk	8.22	320	328	64	210	11	36
	8.28	320	323	69	222	14	44
Rural + Milk	8.39	320	297	68	202	18	54
	8.44	320	301	74	223	23	69

TABLE 11

CHILD PC-204

Age: 2 years 3 months

Height: 80 cms.

Diet	Weight	N. Intake mg/Kg/d		N. Absorbed		N. Retained	
		Planned	Actual	% of Intake	mg/Kg/d	% of Intake	mg/Kg/d
Rural	10.49	256	254	69	176	15	38
	10.60	256	249	63	157	16	40
Rural + Dil. Milk	10.66	320	320	76	243	30	95
	10.78	320	316	75	238	43	135
Rural + Milk	10.94	320	337	84	282	41	138
	11.00	320	338	74	250	29	98

TABLE 12

SUMMARY OF THE STUDY ON THE SUPPLEMENTARY EFFECT OF MILK AND MILK WHOSE ESSENTIAL NITROGEN WAS DILUTED 10%

Diet	No. of Balances	Intake Protein g/Kg/d	Intake Calories Kg/d	Nitrogen Absorption %			Nitrogen Retention %			Nitrogen Retention mg/Kg/d		
				$\bar{X}$	±	S	$\bar{X}$	±	S	$\bar{X}$	±	S
Corn + Black Bean	15	1.6	100	61		6.5	7		12.0	18		30
Corn + Black Bean + Milk	12	1.6 0.4	100	73		5.5	27		6.5	85		24
Corn + Black Bean + Milk Diluted 10%	12	1.6 0.4	100	73		4.4	25		9.4	80		30

Table 13

Nitrogen Balance Data for Young Adult Male Subjects Studied for the Nutritive Value of Dried Skim Milk (DSM) With and Without Supplemented Methionine, and Hen's Egg Protein (Study No. 1)

Subject	N. intake	DSM			DSM + Methionine			Egg		
		Urine	Feces	Balance	Urine	Feces	Balance	Urine	Feces	Balance
		g N per day								
E.A.	2.74	2.45[1]	0.69[1]	-0.40[2]	2.12	0.60	+0.02	2.25	0.77	-0.28
A.C.	3.00	2.25	0.85	-0.10	2.52	0.71	-0.23	2.48	0.76	-0.24
S.E.	3.11	2.61	1.04	-0.54	2.54	0.88	-0.31	2.70	0.95	-0.54
P.G.	2.68	2.57	0.84	-0.74	2.66	0.82	-0.81	2.57	1.14	-1.09
R.G.	3.68	3.62	0.64	-0.58	3.38	0.76	-0.46	3.10	1.00	-0.42
P.J.	2.62	2.64	0.66	-0.68	2.34	0.60	-0.33	2.33	0.92	-0.64
P.L.	3.38	3.98	0.84	-1.44	4.15	0.78	-1.56	3.46	1.10	-1.19
P.N.	2.77	3.34	0.92	-1.49	3.06	0.76	-1.05	2.65	0.83	-0.71

[1] Mean daily value based on last 10 days of each 15-day dietary period.

[2] Intake N - (Urine N-Fecal N)

Table 14

Summary of Derived Values on the Protein Nutritional Quality of Cow's Milk (DSM), Methionine-supplemented DSM and Egg in Young Adult Men[1]

Variable	DSM	DSM + Methionine	Egg
N Balance (gN/day)	-0.75±0.17[2]	-0.62±0.18	-0.63±0.12
"True" Digestibility	93±2	94±2	89±2
Biological Value	87±6	91±6	95±3
Net Protein Utilization	81±6	86±6	87±3

[1]Subjects fed protein at a level equivalent to 0.27g/kg/day.

[2]Mean ± std. error for 8 subjects during the last 10 days of a 15-day experimental period.

The BV and NPU values did not differ ($P > 0.10$ by paired "t" test) between the supplemented DSM and egg protein periods. Methionine supplementation of DSM did not significantly ($P > 0.10$) change BV or NPU values.

Table 15

Nitrogen Balance Data for Young Adult Male Subjects Studied for the Nutritive Value of Dried Skim Milk Protein (DSM), Casein and Lactalbumin (Study No. 3)

Subject	N Intake	DSM			Casein			Lactalbumin		
		Urine	Feces	Balance	Urine	Feces	Balance	Urine	Feces	Balance
		g N per day								
G.D.	3.13	3.47[1]	0.66[1]	-1.0[2]	3.79	0.67	-1.33	2.81	0.58	-0.27
A.P.	3.27	2.95	1.04	-0.73	3.26	0.93	-0.92	2.40	1.05	-0.17
C.S.	2.82	2.80	0.61	-0.59	3.02	0.67	-0.87	2.77	0.68	-0.63
K.S.	3.56	3.06	0.83	-0.31	3.89	0.78	-1.12	2.93	0.88	-0.26
A.V.	2.63	2.74	1.08	-1.19	2.53	0.69	-0.58	3.07	0.63	-1.07
G.W.	2.19	2.06	0.63	-0.51	2.16	0.66	-0.63	1.93	0.66	-0.41

[1] Mean daily value based on last 10 days of each 15-day dietary period.

[2] Intake N - (Urine N - Fecal N).

Table 16

Summary of Derived Values on the Nutritional Quality of Cow's Milk Proteins in Young Adult Men[1]

Variable	Protein Source		
	DSM[2]	Casein	Lactalbumin
N Balance (gN/day)	-0.73±0.13[3]	-0.91±0.12	-0.45±0.14
"True" Digestibility (%)	92±3	94±2	95±2
Biological Value	87±4	79±4	94±6
Net Protein Utilization	80±5	77±3	88±2

[1]Test protein fed at a level equivalent to 0.27g/kg/day (Nx6.25).

[2]DSM-dry nonfat skim milk.

[3]Mean ± std. error for 6 subjects during last 10 days of a 15-day experimental period. Obligatory urine and metabolic fecal N losses assumed to be 37.2 and 8.8mg N per kg body weight per day (Young and Scrimshaw, 1968).

On a paired "test" test basis, the differences between the DSM and lactalbumin values are not statistically significant ($P > 0.1$). If subject AV (see text) is excluded, the BV and NPU of casein are significantly lower than for DSM ($P < 0.01$).

TABLE 17

Urinary nitrogen excretion in young adult men studied for the effects of dilution of milk protein when used as a supplement to a basal wheat-chick pea diet

Subject	Diet		
	Basal	Basal Plus Milk	Basal Plus 20% Dil. Milk
	--------- g N per day ---------		
TA	2.25[1]	2.84	3.43
TB	2.67	3.05	3.16
RH	2.39	3.06	3.27
SS	2.80	3.40	3.72
AW	3.49	4.35	5.36
LW	3.19	3.94	4.09
Mean ± S.D.	2.80 ± 0.47	3.44 ± 0.59	3.84 ± 0.82

1. Mean value for the last 10 days of the 15 day diet period.

TABLE 18

Nitrogen balance for young adult subjects studied for the effects of a 20% dilution of skim milk protein on its supplementary value to a wheat-chick pea diet[1]

Subject	Diet		
	Basal	Plus Skim Milk	Plus Diluted Skim Milk
	--------- g N per day ---------		
TA	-1.14	0.25	-0.15
TB	-1.16	-0.40	-0.54
RH	-1.03	0.30	0.11
SS	-1.42	-0.10	-0.36
AW	-2.28	-1.01	-2.11
LW	-2.16	-0.53	-0.69
Mean ± SD	-1.61±0.52	-0.25±0.50	-0.62±0.78

1. Data based on last 10 days of each diet period.

TABLE 19

Calculated biological values for a wheat-chick pea diet supplemented with skim milk and diluted skim milk when given to young adult male subjects

Subject	Diet		
	Basal	Basal Plus Skim Milk	Basal Plus Dil. Skim Milk
TA	117	91	76
TB	71	73	69
RH	110	90	83
SS	92	81	73
AW	52	56	29
LW	74	71	67

Figure 1

PLAN OF STUDY OF DEPLETED CHILDREN

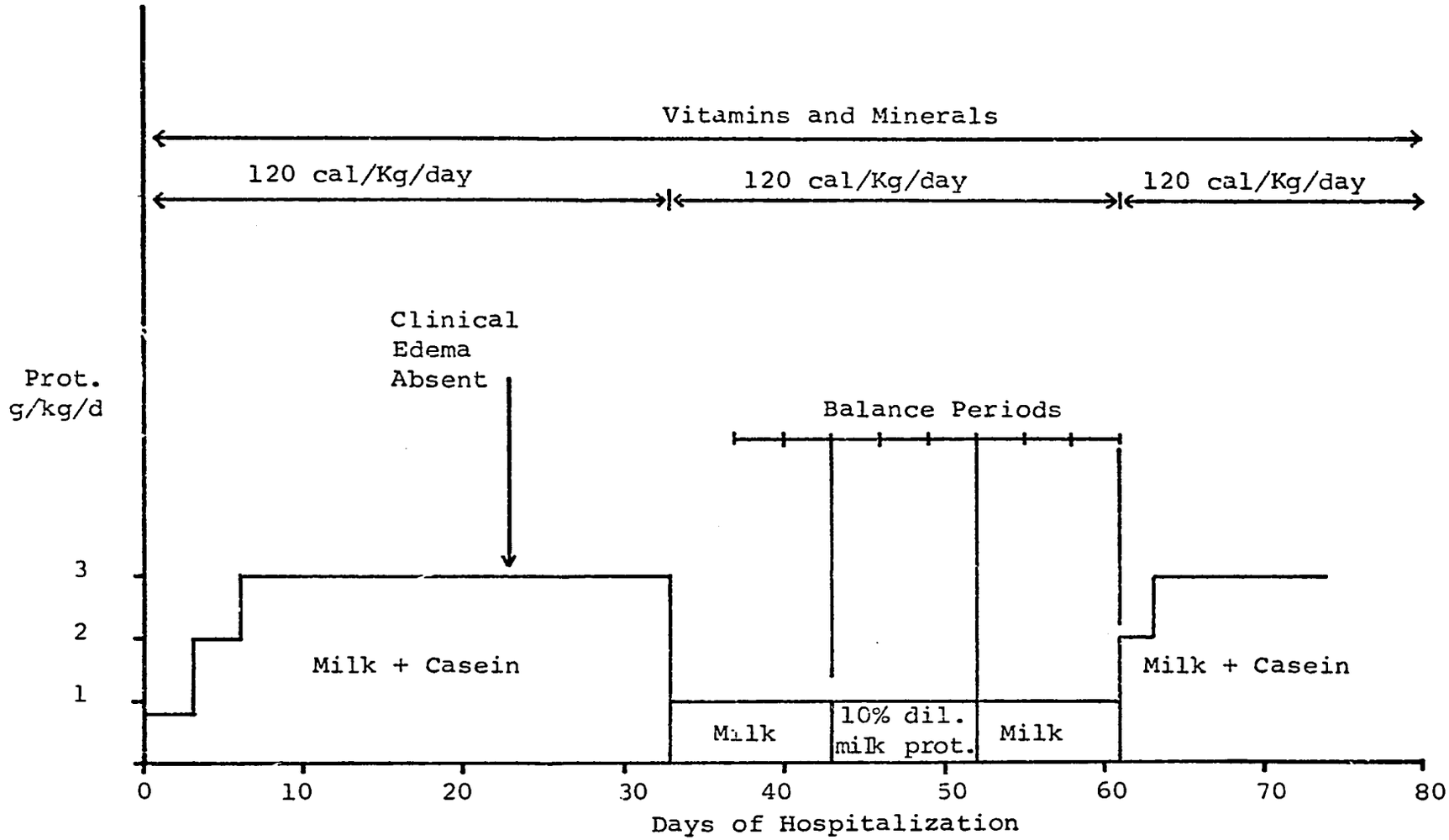


FIGURE 2

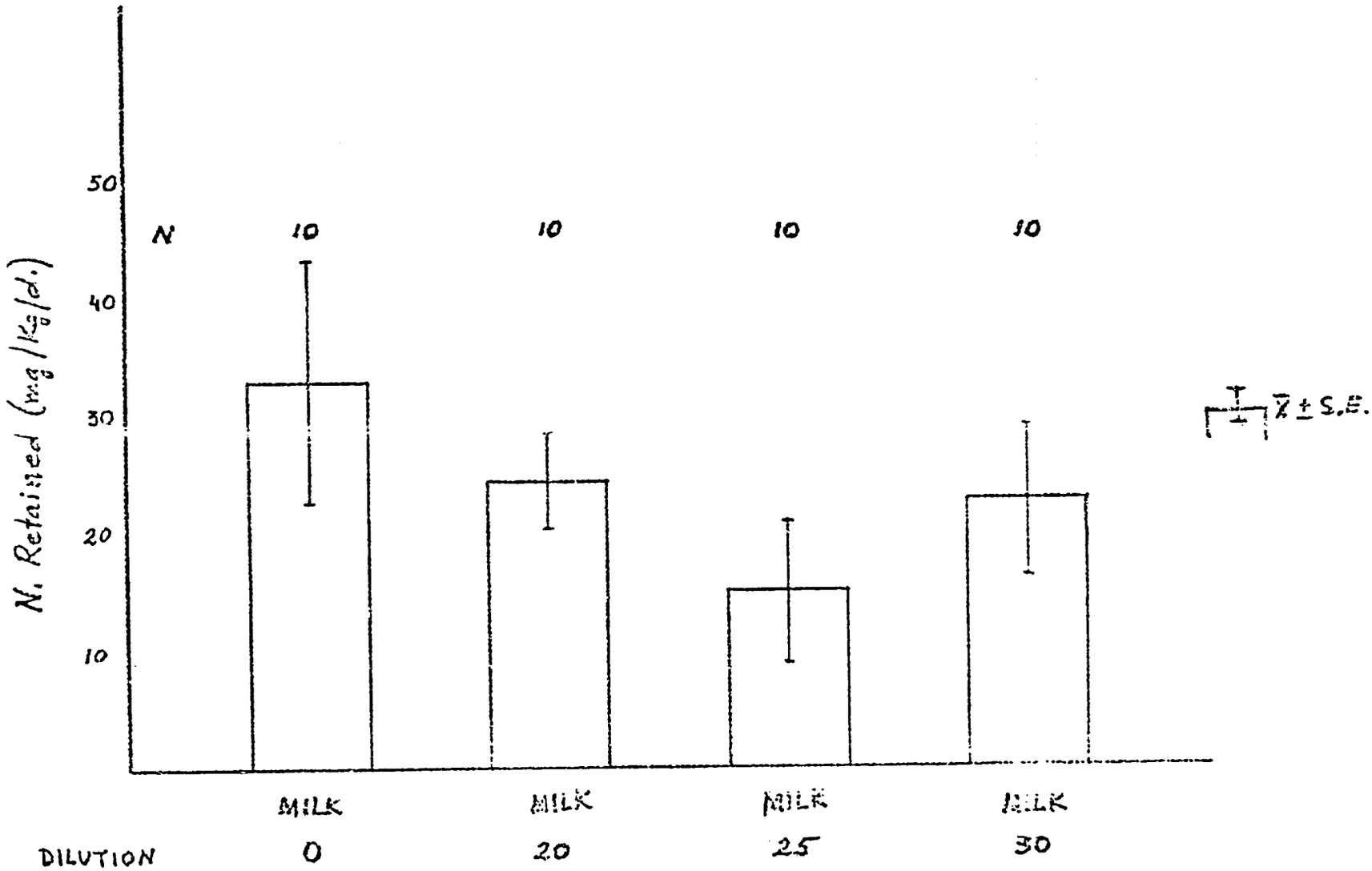

N. Retained (mg/Kg/d.)
50
40
30
20
10
N
10
10
10
10
X̄ ± S.E.
MILK
MILK
MILK
MILK
DILUTION
0
20
25
30

FIGURE 3

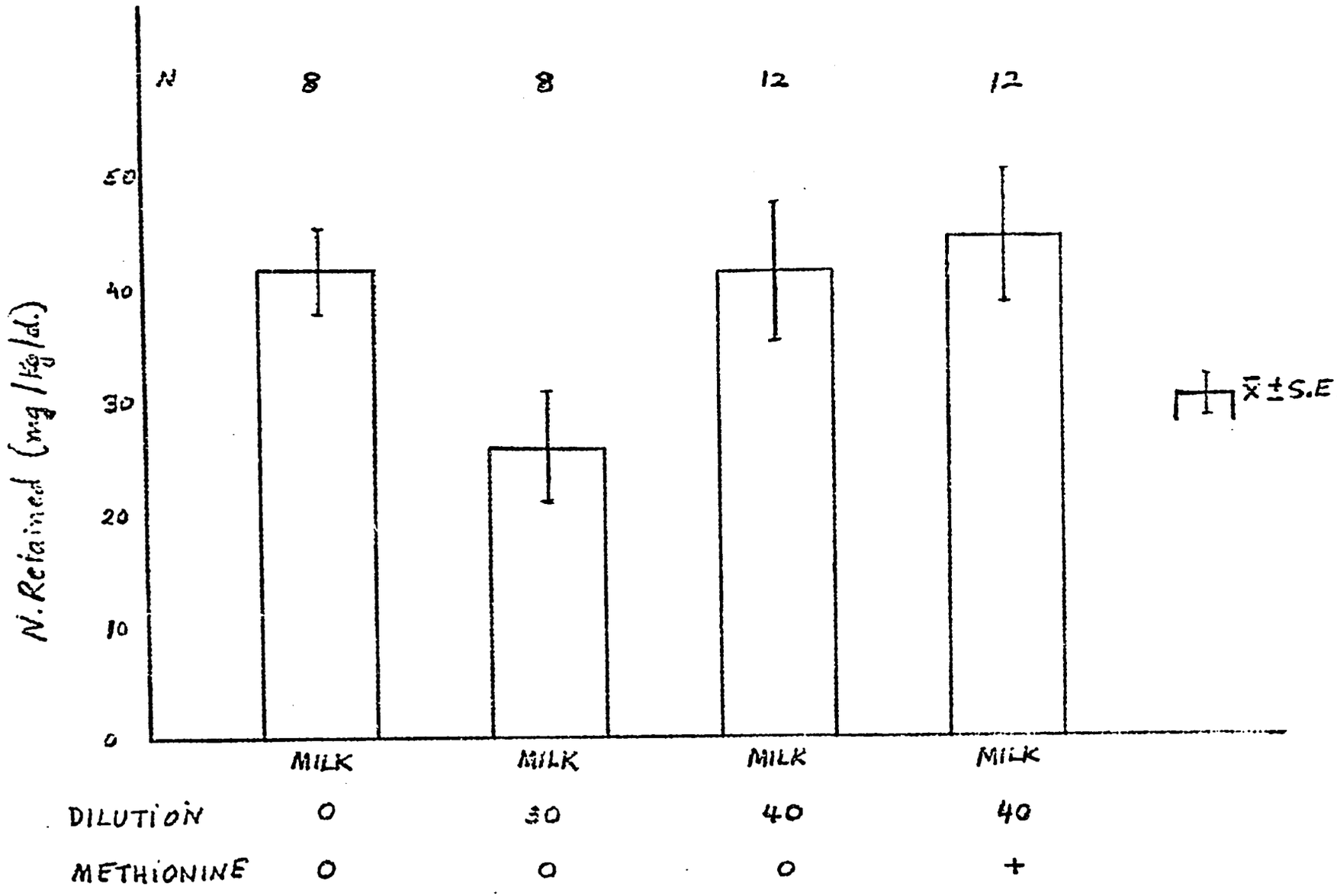

N
8
8
12
12
50
40
30
20
10
0
N. Retained (mg/kg/d.)
MILK
MILK
MILK
MILK
DILUTION
0
30
40
40
METHIONINE
0
0
0
+
x̄ ± S.E.

FIGURE 4

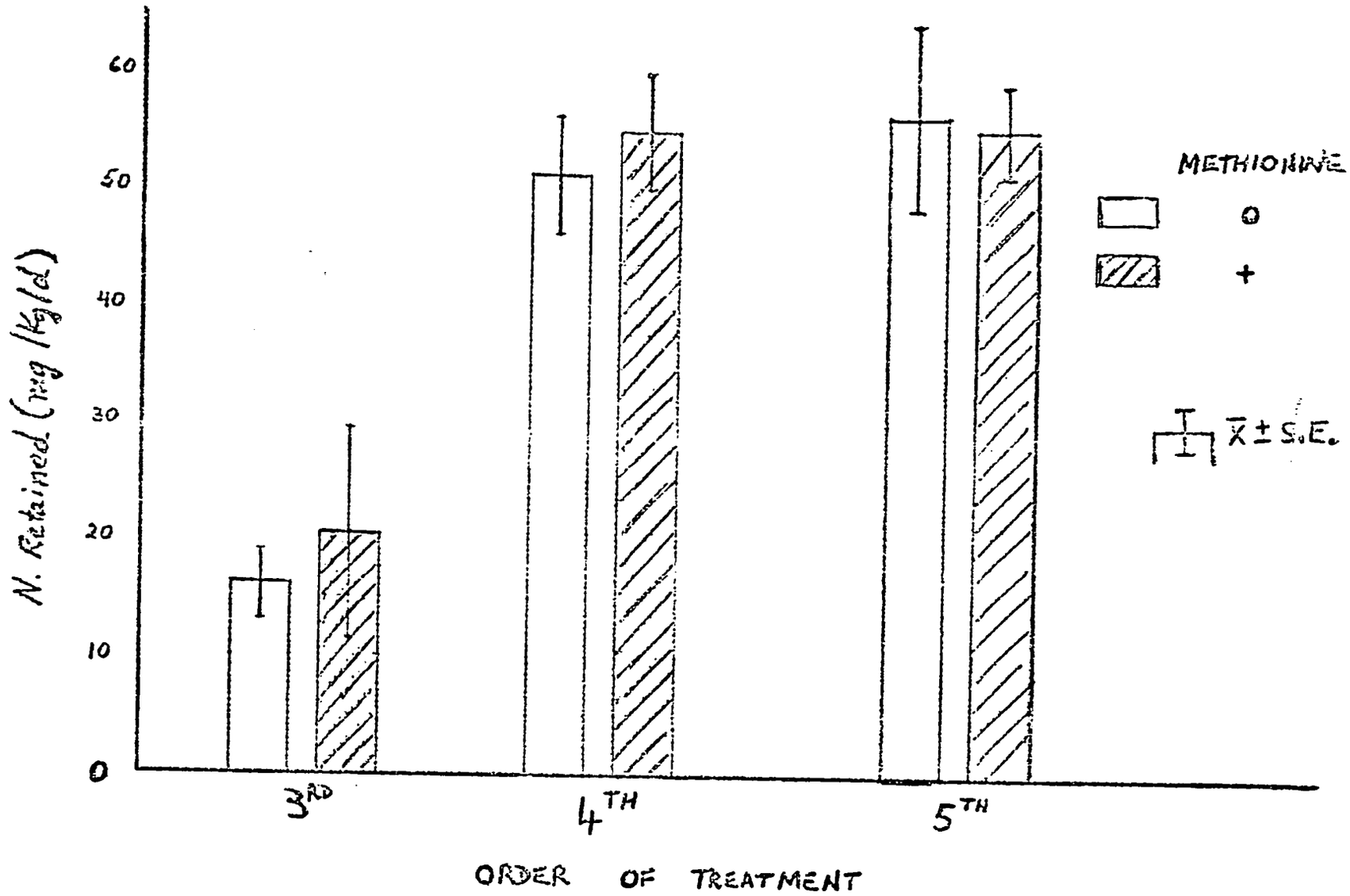
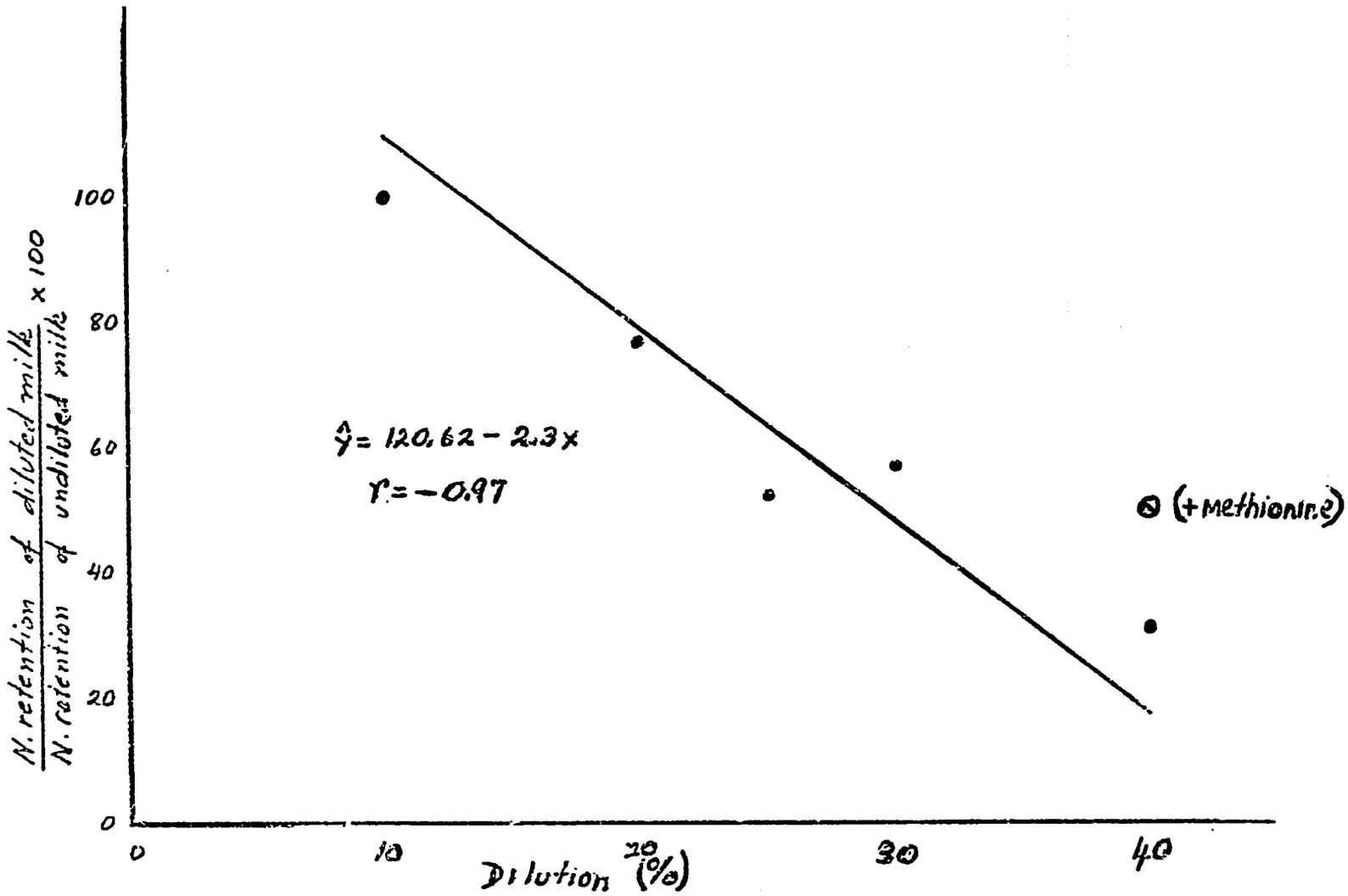

N. Retained (mg/Kg/d)
60
50
40
30
20
10
0
3RD
4TH
5TH
ORDER OF TREATMENT
METHIONINE
0
+
X̄ ± S.E.

FIGURE 5


N. retention of diluted milk / N. retention of undiluted milk × 100
100
80
60
40
20
0
0
10
20
30
40
Dilution (%)
ŷ = 120.62 − 2.3x
r = −0.97
⊗ (+methionine)

APPENDIX I

TABLE 1

CHILD PC-216

AGE 3years-10months

Weight Kg	Height cm	Protein Source	N. Intake Planned mg/Kg/d	N. Intake Real mg/Kg/d	N. Absorbed mg/Kg/d	N. Absorbed %	N. Retaine mg/Kg/d	N. Retaine %
13.61	94.3	Milk	160	160	116	72	26	16
13.76	95.0	Milk+20%Glyc	160	166	128	77	5	3
13.76	95.0	Milk+20%Glyc	160	156	141	90	35	28
13.95	95.0	Milk+25%Glyc	160	151	125	82	-19	-13
13.91	95.0	Milk+25%Glyc	160	151	93	62	- 8	- 5
14.10	95.0	Milk+30%Glyc	160	152	121	80	22	12
14.25	95.0	Milk+30%Glyc	160	157	127	81	23	14

APPENDIX I

TABLE 2

CHILD PC-217

AGE 5 years-11 months

Weight Kg	Height cm	Protein Source	N. intake Planned mg/Kg/d	N. intake Real mg/Kg/d	N. absorbed mg/Kg/d	N. absorbed %	N. retained mg/Kg/d	N. retained %
22.64	100	Milk	160	149	113	75	57	38
22.04	100	Milk	160	156	117	75	44	28
22.07	101	Milk+20%Glyc	160	151	127	84	38	25
22.23	101	Milk+20%Glyc	160	167	127	76	38	22
22.60	101	Milk+25%Glyc	160	147	116	79	37	25
23.02	101	Milk+25%Glyc	160	162	114	70	38	28
23.07	101	Milk+30%Glyc	160	160	136	85	73	45
23.10	101	Milk+30%Glyc	160	178	143	80	27	18

APPENDIX I

TABLE 3

CHILD PC-220

AGE 4 years-4 months

Weight Kg	Height cm	Protein Source	N. Intake Planned mg/Kg/d	N. Intake Real mg/Kg/d	N. Absorbed mg/Kg/d	N. Absorbed %	N. Retained mg/Kg/d	N. Retained %
15.76	96	Milk	160	157	77	49	-18	-11
15.76	96	Milk	160	163	134	82	57	35
15.76	96	Milk+20%Glyc	160	158	119	76	11	7
15.72	96	Milk+20%Glyc	160	154	116	76	13	9
15.69	96	Milk+25%Glyc	160	158	117	74	13	8
15.65	96	Milk+25%Glyc	160	159	105	66	6	4
15.76	96	Milk+30%Glyc	160	154	105	68	- 3	- 3
15.76	97	Milk+30%Glyc	160	168	130	77	18	10

APPENDIX I

TABLE 4

CHILD PC-222

AGE 5 years-6 months

Weight Kg	Height cm	Protein Source	N. Intake Planned mg/Kg/d	N. Intake Real mg/Kg/d	N. Absorbed mg/Kg/d	N. Absorbed %	N. Retained mg/Kg/d	N. Retained %
14.55	96	Milk	160	155	133	86	57	37
14.67	96	Milk	160	154	113	73	38	24
14.29	96	Milk+20%Glyc	160	156	140	89	35	22
14.36	96	Milk+20%Glyc	160	162	120	74	22	13
14.29	96	Milk+25%Glyc	160	158	116	73	20	12
14.29	96	Milk+25%Glyc	160	165	145	88	20	12
14.36	97	Milk+30%Glyc	160	157	118	75	17	11
14.29	97	Milk+30%Glyc	160	158	130	75	25	15

APPENDIX I

TABLE 5

CHILD PC-226

AGE 3 years-1 month

Weight Kg	Height cm	Protein Source	N. Intake Planned mg/Kg/d	N. Intake Real mg/Kg/d	N. Absorbed mg/Kg/d	N. Absorbed %	N. Retained mg/Kg/d	N. Retained %
11.83	76	Milk	160	158	93	59	26	17
11.79	76	Milk	160	175	131	75	69	40
11.87	77	Milk+20%Glyc	160	160	111	69	22	14
11.83	77	Milk+20%Glyc	160	164	118	72	21	18
11.91	77	Milk+25%Glyc	160	166	125	75	32	19
11.94	77	Milk+25%Glyc	160	162	110	68	12	7
11.98	77	Milk+30%Glyc	160	160	113	71	19	12
12.04	77	Milk+30%Glyc	160	157	108	68	6	4

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TABLE 6

CHILD PC-221

AGE 2 years-4 months

Weight Kg	Height cm	Protein Source	N. Intake Planned mg/Kg/d	N. Intake Real mg/Kg/d	N. Absorbed mg/Kg/d	N. Absorbed %	N. Retained mg/Kg/d	N. Retained %
14.36	88	Milk	160	163	134	82	52	32
14.67	88	Milk	160	150	110	73	36	24
14.74	88	Milk+30%Glyc	160	158	122	78	23	15
14.82	88	Milk+30%Glyc	160	157	128	82	20	13
14.86	90	Milk+40%Glyc	160	161	123	76	10	6
14.93	90	Milk+40%Glyc	160	152	117	76	14	9
14.78	90	Milk+40%Glyc+Meth	160	155	118	76	47	30
14.97	90	Milk+40%Glyc+Meth	160	163	130	82	70	44
15.01	90	Milk+40%Glyc	160	155	130	84	64	41
15.08	90	Milk+40%Glyc	160	163	133	82	71	44

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TABLE 7

CHILD PC-226

AGE 3 years-3 months

Weight Kg	Height cm	Protein Source	N. Intake Planned mg/Kg/d	N. Intake Real mg/Kg/d	N. Absorbed mg/Kg/d	N. Absorbed %	N. Retained mg/Kg/d	N. Retained %
12.55	78	Milk	160	157	103	65	35	22
12.55	78	Milk	160	158	104	66	41	26
12.70	78	Milk+30%Glyc	160	160	114	71	20	13
12.81	78	Milk+30%Glyc	160	158	99	62	1	0
12.81	80	Milk+40%Glyc+Meth	160	160	121	76	16	10
12.81	80	Milk+40%Glyc+Meth	160	162	111	68	- 1	- 1
12.85	80	Milk+40%Glyc	160	160	121	75	50	31
12.93	80	Milk+40%Glyc	160	150	110	73	41	27
12.97	80	Milk+40%Glyc+Meth	160	157	117	74	51	33
13.04	80	Milk+40%Glyc+Meth	160	154	118	77	47	31

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TABLE 8

CHILD PC-229

AGE 4 years-7 months

Weight Kg	Height cm	Protein Source	N. Intake Planned mg/Kg/d	N. Intake Real mg/Kg/d	N. Absorbed mg/Kg/d	N. Absorbed %	N. Retained mg/Kg/d	N. Retained %
15.20	88	Milk	160	159	95	60	24	15
15.38	88	Milk	160	158	102	66	37	24
15.08	88	Milk+30%Glyc	160	152	118	78	24	16
15.08	90	Milk+30%Glyc	160	155	118	76	30	19
15.16	90	Milk+40%Glyc	160	153	124	81	22	14
15.08	90	Milk+40%Glyc	160	150	117	78	18	12
15.08	90	Milk+40%Glyc+Meth	160	153	112	73	51	33
15.01	90	Milk+40%Glyc+Meth	160	152	119	78	51	34
15.01	90	Milk+40%Glyc	160	153	116	76	56	37
14.93	90	Milk+40%Glyc	160	154	122	79	34	22

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TABLE 9

CHILD PC-230

AGE 2 years-4 months

Weight Kg	Height cm	Protein Source	N. Intake Planned mg/Kg/d	N. Intake Real mg/Kg/d	N. Absorbed mg/Kg/d	N. Absorbed %	N. Retained mg/Kg/d	N. Retained %
11.78	78	Milk	160	163	128	78	56	35
11.79	78	Milk	160	158	131	83	51	32
11.75	78	Milk+30%Glyc	160	152	122	80	41	27
11.75	78	Milk+30%Glyc	160	157	134	86	46	29
11.79	81	Milk+40%Glyc+Meth	160	155	126	81	26	17
11.75	81	Milk+40%Glyc+Meth	160	161	144	89	41	26
11.72	81	Milk+40%Glyc	160	138	119	86	66	48
11.57	81	Milk+40%Glyc	160	151	122	81	49	32
11.68	81	Milk+40%Glyc+Meth	160	158	126	80	66	42
11.68	81	Milk+40%Glyc+Meth	160	156	131	84	56	36

APPENDIX 2

Summary of Findings in Limited Rat Studies

A number of experiments studied growth of weanling rats and urinary nitrogen excretion in young adult rats when cow's milk protein was partially replaced by varying levels of supplementary nitrogen. A mixture of nonessential L-amino acids (NEAA) or a mixture of diammonium citrate and glycine (DAC-Gly) was used as the source of nitrogen. Substitution of the 15% milk protein diet to the extent of 10% slightly reduced growth; significant growth reduction occurred with substitutions of 20% and greater. Fortification of the diets containing 10.5% milk protein and the supplementary nitrogen sources with sulfur amino acids did not restore growth to the maximum rate obtained with the 15% milk protein diet. Additional supplementation with tryptophan further improved growth slightly but not to the maximum rate. Several other essential amino acids, alone or in combination, had no apparent effect. Based on urinary nitrogen excretion, comparable results were obtained with young adult rats by substituting nitrogen for milk protein in the diets. The reduced performance following substitution of milk protein with supplementary nitrogen may be due partly to decreased utilization of sulfur amino acids and possibly to decreased utilization of all essential amino acids. (Published in J. Food Sci. 35:170 (1970))